O.

NOV 10 1952

SCIENCE
LIBRARY

VOLUME 42 OCTOBER, 1952 NUMBER 10

Phytopathology

An International Journal

Official Organ of

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

CONTENTS

Life cycle of Botryosphaeria ribis on Aleurites montana ..... P. O. Wiehe 521

Factors affecting the survival ability of certain physiologic races of Puccinia graminis avenae Erikss. & Henn ..... M. K. Hingorani 526

Mycelial mats of Chalara quercina on killed oak trees as a potential source of perithecia in nature (A phytopathological note)
H. L. Barnett, John M. Staley, and R. P. True 531

Six new leafhopper vectors of Pierce's disease virus
H. L. Freitag, N. W. Frazier, and R. A. Flock 533

Vein clearing on sugar beet induced by the beet leafhopper ..... C. W. Bennett 535

A mechanism for liberation of spores from beneath the bark and for diploidization in Chalara quercina ..... J. G. Leach, R. P. True, and C. K. Dorsey 537

Aphid transmission of potato leafroll virus to solanaceous species
Hugh C. Kirkpatrick and A. Frank Ross 540

Black thread of Hevea brasiliensis in Liberia
Ellis F. Darley and Savel B. Silverborg 547

The effect of smog on the anatomy of oat leaves ..... Ruth Ann Bobrov 558

Altering resistance to disease with synthetic organic chemicals
David Davis and A. E. Dimond 563

Discoloration of soybean seeds by the frogeye fungus, Cercospora sojina
Helen S. Sherwin and K. W. Kreitlow 568

Common lettuce mosaic and its control by the use of mosaic-free seed
R. G. Grogan, J. E. Welch and Roy Bardin 573

Sori of gladiolus smut in the white flesh of gladiolus corms (A phytopathological note)
Donald P. Limber 578

News

Subscription, $10.00 per year

Office of Publication—Monumental Printing Company, Baltimore, Md.

Entered as second-class matter at the post office at Baltimore, Md.

DuPont Announces

MANZATE

TRADE MARK

FUNGICIDE

for TOMATOES and POTATOES

Based on Manganese Ethylene Bisdithiocarbamate

EXPERIMENTAL QUANTITIES NOW AVAILABLE

"Manzate", sixth in a series of dithiocarbamate fungicides offered by Du Pont, has special advantages on tomatoes and potatoes. It has already given a highly satisfactory account of itself in many states this year where it has been evaluated in carefully conducted tests and has also been used by commercial growers in all the principal tomato producing states. This substantiates results previously obtained in several years' tests in 32 states, Hawaii, Canada and Mexico.

FOR TOMATOES—"Manzate" fungicide is a single material effective against all the major fungous diseases of tomatoes. Now a program is offered employing "Manzate" alone to control diseases instead of alternate chemicals in separate sprays. "Manzate" is effective against early and late blight, anthracnose, gray leaf spot (Stemphylium), and Septoria leaf spot.

FOR POTATOES—"Manzate" is particularly effective against both early blight and late blight, especially when both these diseases are severe at the same time.

At present, Du Pont's recommendations for use of "Manzate" apply only to tomatoes and potatoes. As a result of numerous tests conducted during the past season on certain other crops it appears that "Manzate" will have a much wider range of application. Experimental quantities of "Manzate" fungicide are now available for research purposes. "Manzate" will be tested still further on additional crops in 1953. For further information, write Du Pont, Grasselli Chemicals Department, Wilmington, Delaware.

DU PONT

REG. U.S. PAT. OFF.

BETTER THINGS FOR BETTER LIVING
. . . *THROUGH CHEMISTRY*

LIFE CYCLE OF BOTRYOSPHAERIA RIBIS ON ALEURITES MONTANA [1]

P. O. Wiehe [2]

Dieback of the tung oil tree, *Aleurites montana* Forsk., caused by *Botryosphaeria ribis* Gross. & Dug., is one of the serious diseases to which this crop is subjected in Nyasaland. The disease was first reported (2) in 1939 under the name of branch and twig canker caused by *Dothiorella* sp., but was not studied in great detail until 1949. In view of the potential threat of dieback to the growing tung oil industry of Nyasaland, some time was devoted during 1949 and 1950 to the study of this disease with the object of formulating control measures based on a knowledge of the life cycle of the organism.

During these investigations, *Botryosphaeria ribis* was repeatedly isolated from infected tissues. Inoculations into healthy plants produced typical cankers and dieback, and fructifications of the fungus with the perfect and imperfect stages were obtained both on the bark of dead branches and in culture. Materials authoritatively identified include the following collections: No. 220, isolated from dying seedling (I.M.I. 36476); No. 489, isolation from infected male inflorescence (I.M.I. 39582); No. 558, isolation from fruit (I.M.I. 43081); No. 813, fruiting bodies on bark of dead branch with pycnospores and ascospores (I.M.I. 43082); No. 818, diseased twigs with pycnospores (I.M.I. 43803).

Botryosphaeria ribis has been previously reported as the cause of canker and nut rot on *A. fordii* in the United States (6, 8, 9) and also on *A. molucanna* in the Hawaiian islands (13). The fungus is known to attack a large number of woody species belonging to different families in tropical and subtropical regions (12, 13). In Nyasaland, *Botryosphaeria ribis* has caused dieback and canker of *Aleurites montana*, *A. fordii*, *Camellia theae* grown for seeds, *Godetia* sp., *Grevillea robusta*, *Manihot utilissima*, *Persea americana*, *Psidium guajava* and *Pyrus malus*.

The disease is widespread and occurs throughout the tung-growing areas but is more severe in the Cholo district. The fungus causes a blight of young shoots and dieback of large branches, seedlings, and young budded stocks. In rare cases the fruits are directly infected. *Botryosphaeria ribis* may also cause a stump rot following sunscorch injury (15).

[1] Accepted for publication March 10, 1952.

The author is indebted to Mr. E. W. Mason and Dr. M. B. Ellis of the Commonwealth Mycological Institute, for examining and authoritatively identifying a number of cultures and specimens. Dr. G. R. Bates, Senior Pathologist, Southern Rhodesia, suggested in the first place, the possibility of latent infection of the fruits, and otherwise contributed to this investigation by other valuable suggestions. Finally the writer is most grateful to Dr. C. C. Webster, formerly Senior Agricultural Officer, Tung Station and Mr. J. A. Sandys for their valuable assistance in many ways on the field aspect of this investigation.

[2] Plant Pathologist, Department of Agriculture, Nyasaland.

The symptoms of dieback on *A. montana* have already been described (14, 15). Briefly, they include a wilt or blight of young shoots common during the earlier part of the growing season and in which primary infection can be traced to fruit stalks of the preceding season or to the terminal bud. This may be followed by wilt of large branches, accompanied by cankers which take the form of longitudinal splits of the bark, usually on one side of the branch. Infection in the latter case has occurred several seasons previously but the presence of the pathogen becomes apparent only when girdling of the stem has taken place. Dieback of large branches may also result from penetration of the fungus through wounds on the stem.

INVESTIGATIONS ON THE MODE OF INFECTION OF B. RIBIS.—During a study of dieback incidence in an experimental orchard at the Tung Station, Cholo, which included a survey of the disease status of 360 trees [3] it was found that in 51 per cent of the cases examined primary infection was related to the fruit stalks of the preceding season (Fig. 1, B), and that infection apparently arising from the terminal bud was recorded in 38 per cent of the dying shoots (Fig. 1, A). The diagnostic feature selected was the presence of stromata-bearing spores of *Dothiorella* type all along the fruiting twig above the point of origin of the wilting lateral shoot (Fig. 2). As fruiting bodies were not observed on the bark of the dying shoot except at the point of branching, it was concluded that the fungus had grown from the fruit stalks or the apical bud, killing the lateral shoots in its downward invasion of the stem. In order to verify these observations, experiments were designed with a view of determining when primary infection of the fruit stalks took place; they are reviewed below in their logical order.

Cultural characteristics.—Although the characteristics of *B. ribis* in culture have been fully described by previous workers (4, 10, 11), some of the results obtained during the investigations described in this paper are at variance with accepted views. More particularly was this found to be the case in connection with chromogenicity of the fungus in culture and its parasitism. Thus, of 20 isolates grown on cornmeal agar, 17 cultures were chromogenic and 3 did not produce a distinctive color on the media. Coloration was determined after 5 and 10 days' growth by comparison with Munsell's standard color charts. Chromogenic cultures turned the media yellowish green at first, becoming gradually reddish yellow. The 3 non-chromogenic cultures which had originally been isolated from dying stems of *Aleurites montana* were reinoculated on seedlings. The inoculations were positive, producing dieback with spores of *Dothiorella gregaria*

[3] This survey was carried out by Mr. J. A. Sandys, Agricultural Officer, Tung Experimental Station, to whom the author is greatly indebted for permission to use these data.

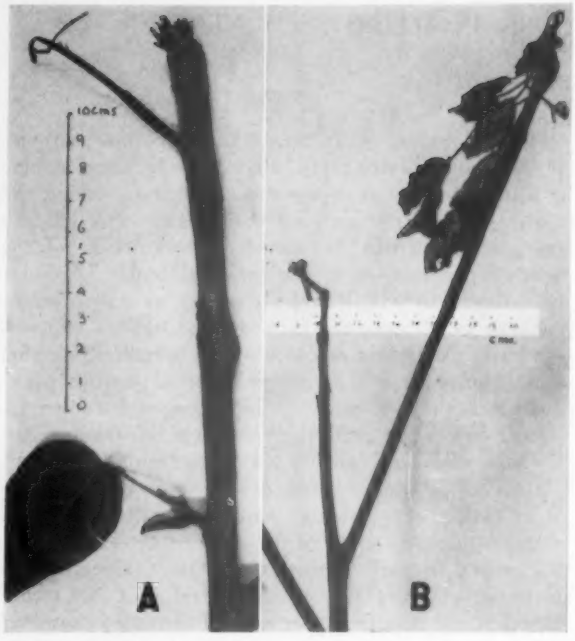


FIG. 1. Dieback of *Aleurites montana* caused by *Botryosphaeria ribis* Gross. & Dug. A, primary infection arising from apex of stem, the fungus having killed a recently developed lateral shoot to the left. B, the fungus has invaded the tissues from the fruit stalk killing a lateral shoot in its progress down the stem. (Photo by P. R. O., Nyasaland.)

Sacc. 5 weeks after inoculation. These results indicate that parasitism in *Botryosphaeria ribis* is not always associated with chromogenesis in culture. Consequently, no further attention was paid to color development of isolations during routine work connected with the life history studies; instead, an attempt was made to devise culture methods whereby spores would be quickly obtained in culture. Various media were tried including malt, prune, cornmeal, and potato-carrot extracts, none of which proved satisfactory in either tubes or plates. On malt agar many stromata were often produced, but were generally sterile. The fungus was next grown on sterilized avocado and tung twigs in tubes with an without approximately 3 cc. of malt agar. Addition of malt agar to the tubes greatly favored sporulation, which usually started about 3 weeks after inoculation. The size of the stromata varied according to the types of twigs used, being consistently larger on tung than on avocado. *Botryodiplodia theobromae* Pat. was often isolated during the course of investigations on tung diseases and some difficulties were encountered in distinguishing between the vegetative characteristics of the 2 species in culture. On sterile twigs, however, the mycelium was generally more fluffy, with a typical purplish tinge absent from cultures of *Botryosphaeria ribis*. In addition spores of *Botryodiplodia* developed much sooner and pycnidia were often produced on plain malt agar.

Surface mycoflora of fruits.—With a view to determining the species of fungi which might be present on the surface of fruits at various stages of development, single fruits were detached and placed immediately in sterile tubes in the field. Dilution cultures were made about 18 hr. later by shaking the fruits with 10 cc. of sterile water, inoculating 2 drops of the suspension in 5 cc. acidified malt or cornmeal agar, and plating out. Ten plates were prepared approximately at monthly intervals during October, November, and December, the size of the fruits ranging from about 1 cm. along the vertical axis in October to about 3 cm. in December. Some plates were so densely seeded that it was not possible to transfer individual colonies. Common molds (*Aspergillus, Rhizopus, Penicillium, Cladosporium*) were avoided. A total of 28 cultures was finally obtained and the frequency of species isolated is shown in Table 1.

Location of spores on the fruits.—In order to determine the location of the varied mycoflora, including potential pathogens, on the fruit surface at various seasons, young fruits were removed aseptically and placed immediately in sterile dry tubes. The fruits were sterilized individually first in a saturated borax solution as a wetting agent for 10 min., and for 5 min. in alcoholic mercuric chloride; they were then

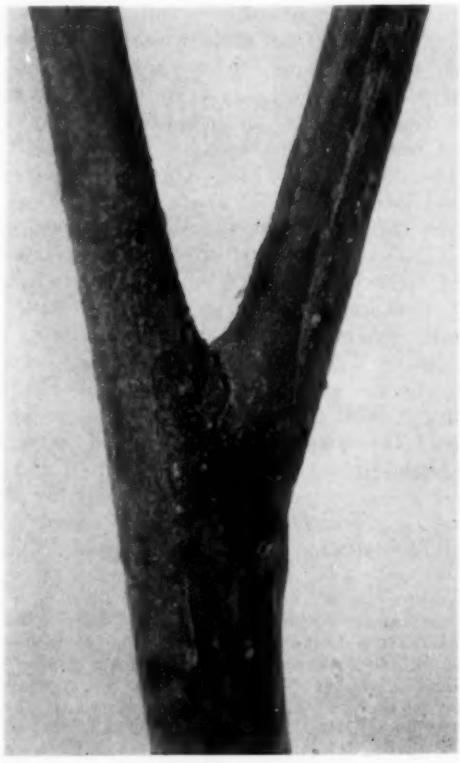


FIG. 2. Close-up photograph of shoot in figure 1, showing development of fruiting bodies along the fruit twig and at the base of the wilting lateral shoot at right. Note the splitting bark, a common feature of the disease after death of the twig. (Photo by P. R. O., Nyasaland.)

TABLE 1.—*Species isolated from fruit surface of Aleurites montana*

	Number of colonies		
	October	November	December
Botryodiplodia theobromae Pat.	2	0	4
Botryosphaeria ribis Gross & Dug.	1	2	2
Alternaria sp.	1	1	0
Gloeosporium sp.	0	2	0
Pestalotia versicolor Speg.	0	0	1
Unidentified species	4	3	5
	8	8	12

thoroughly washed in sterile water and planted in malt agar. Of the 10 fruits cultured 11 colonies were isolated, the transfers being made as soon as sufficient vegetative growth of the mycelium had taken place on the culture media. Three colonies, which all proved to be *Aspergillus* sp., developed from the stigma end of 3 fruits; all the others, including 3 *Alternaria*, 2 *Aspergilli*, 3 *Dothiorella* and 2 *Fusaria*, arose from the base of the fruit near the perianth scar.

These colonies might have arisen either from mycelium within the fruit tissue or from spores located on a portion of the fruit, which had not been in contact with the disinfectant. It is difficult to explain the presence of mycelium of saprophytic species such as *Aspergillus*, *Alternaria*, and possibly *Fusarium* within the actively developing tissue of young fruits. On the other hand the presence of the pathogenic species *Botryosphaeria ribis* within the tissues would normally result in externally visible lesions, but such was not the case. In view of these results longitudinal sections of young fruits were prepared to study the structure of the basal portion. It was found that the perianth scar forms a cavity approximately 1 mm. in depth with a rough corky surface about 100 μ thick. This cavity is protected by the outgrowing pericarp which itself is densely clothed with hairs in the young stages of fruit development.

Although further investigations are desirable, the view is held that the colonies obtained in culture in the experiment under reference arose from spores lodged in the cavity of the perianth scar, where they were protected from contact with the sterilizing solution.

Penetration of the fungus into fruit stalks.—As a result of the observations recorded above, further isolations were made on progressively older fruits using only the region of the perianth scar and a section of the peduncle immediately below the fruit. The material was collected singly in sterile containers, the selected portions being cut aseptically and disinfected in borax and mercuric chloride. Five fruits were used in November, 8 in December, and 4 in February. The results obtained are shown in Table 2..

It is significant that *Botryosphaeria ribis* was isolated in every series of cultures from the region of the perianth scar, thus confirming the results expressed in Table 1. Further, the fact that 1 colony was obtained from the fruit stalk affords positive evidence that the fungus may penetrate the plant tissues sometime before fruit fall.

Peduncular infection.—Soon after fruit fall, in the first week of June, 41 fruit twigs bearing 190 fruit stalks were removed at random from a group of 4 trees which had shown Botryosphaeria infection the preceding season. They were examined in the laboratory and isolations made from 33 peduncles and 11 twigs suspected of being infected. The results obtained are in Table 3.

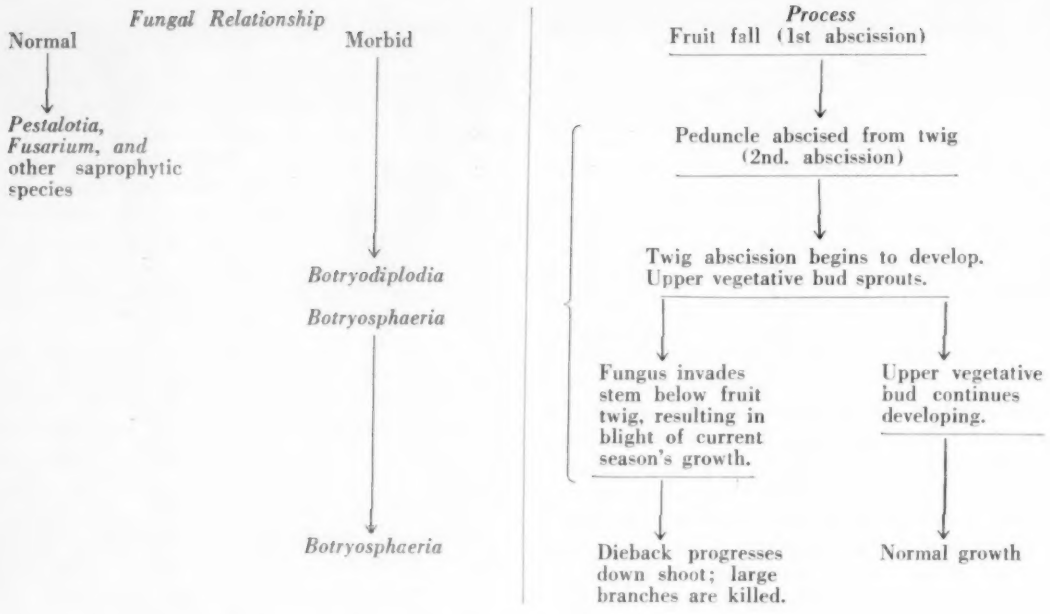
Three abscission layers are responsible in *Aleurites montana* for the processes of fruit fall and isolating the old fruit twig from the stem. The first layer develops about 5 mm. below the bud and severs the fruit from the peduncle; the second is formed at the base of the fruit stalk and fruit twig; and the third develops above the upper vegetative bud. In twig abscission there is first suberization of the parenchymatous cells and gum occlusion of the vessels to form a plate across the stem. This is followed by cork formation, which arises laterally from the phelloderm of the bark and develops inwards below the plate of sclerenchymatous cells, cutting off the dead twig from the lower portion of the stem. The first and second abscission layers normally develop rapidly, whereas twig abscission may take 2 years to be completed. It is normal to expect the development of a saprophytic flora on the senescent peduncles and fruit twigs, but the results of isolations show that potential pathogens may also be present. In this case, therefore, infection of healthy tissues will depend upon the rate at which the fungus grows in the

TABLE 2.—*Mycoflora of perianth scar and fruit stalks of Aleurites montana*

Month	Species and number of colonies isolated from: Perianth scar		Peduncle	
November	*Botryosphaeria*	1	Negative	
	Pestalotia	1		
	Penicillium	3		
December	*Botryosphaeria*	2	Negative	
	Cladosporium	1		
February	*Botryosphaeria*	1	Botryosphaeria	1
	Fusarium	1		
	Botryodiplodia	1		

TABLE 3.—*Peduncular infection after fruit fall*

	Isolation from: Twig	Peduncles
Botryosphaeria ribis Gross & Dug.	6	10
Botryodiplodia theobromae Pat.	2	2
Pestalotia versicolor Speg.	3	11
Fusarium sp.	0	1
Negative	0	9
Total	11	33


Fungal Relationship
Normal
Morbid
Pestalotia, Fusarium, and other saprophytic species
Botryodiplodia
Botryosphaeria
Botryosphaeria
Process
Fruit fall (1st abscission)
Peduncle abscised from twig (2nd. abscission)
Twig abscission begins to develop. Upper vegetative bud sprouts.
Fungus invades stem below fruit twig, resulting in blight of current season's growth.
Upper vegetative bud continues developing.
Dieback progresses down shoot; large branches are killed.
Normal growth

plant tissues compared with the rate at which an effective barrier is being formed across the stem. In addition to twig blight caused by *Botryosphaeria ribis*, it has been found that *Botryodiplodia theobromae* also frequently kills young lateral shoots by invading the stem tissues below the twig abscission. This fungus has already been reported on *Aleurites* spp. by various workers (1, 3, 5, 7); Large (6) has described a girdling of branches in *Aleurites fordii* caused by *Botryodiplodia theobromae*. In Nyasaland, in 180 cases of twig blight, *Botryodiplodia* pycnidia were present on 48 branches; the other twigs bore pycnospores of *Botryosphaeria ribis*. At this stage further advance of Botryodiplodia infection down the stem appears to be effectively checked and no cases of dieback of branches which could be attributed to this cause were found. On the other hand *Botryosphaeria ribis*, once present in the tissues, continues invading the stem causing the ultimate death of large branches. The process of twig abscission in relation to fungal development and parasitism is summarized in the diagram above.

Seasonal incidence of dieback and development of fruiting bodies.—In order to determine the actual incidence of dieback resulting from fruit stalk infection, 50 apparently healthy twigs bearing fruit clusters were labeled serially on 1 tree just before fruit fall in May 1950. Observations on their subsequent history were made at intervals thereafter. The results obtained (Table 4) show that blight of the current season's shoot arising from peduncular infection follows a typical sigmoidal curve reaching its highest incidence in October, about 4 months after fruit fall. These data also indicate that infection had taken place at approximately the same time, the fungus progressing at the same rate down the fruiting twig and into the stem below the upper vegetative shoot.

Fruiting bodies begin to burst through the bark of the dead twigs about 1 mo. after the shoots have been killed. Considerable variation was observed with regard to their density per unit area and the size of the stromata and number of chambers within them.

The stromata are black, roughly elliptical, immersed, and spreading under the bark. Size range of 40 stromata after removal of the bark was: 0.2 — 0.8 × 0.1-0.5 mm. (0.5 × 0.4) mm.

Pycnospores are produced early in the season and are followed about 4 months later by development of asci, but no explanation can be offered on the significance of this observation. Spore dimension averaged 13-25 × 6-10 (19 × 7) μ for ascospores (40 spores

TABLE 4.—*Incidence of dieback*

Date of observation	Number of shoots affected	Fruiting bodies
1950		
May 17	Twigs selected for observation	
June 26	0	Nil
Aug. 17	1	Nil
Sept. 12	2	Nil
Oct. 4	8	Rare, beginning to form
Oct. 20	10	Rare, pycnospores only
Nov. 15	10	Abundant pycnospores only
Dec. 4	10	Abundant pycnospores only
1951		
Jan. 30	10	Abundant pycnospores and ascospores

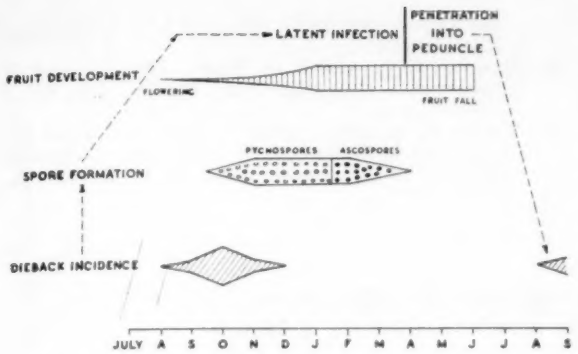


FIG. 3. Diagrammatic representation of the life cycle of *Botryosphaeria ribis* Gross. & Dug. on *Aleurites montana* (explanation in text).

from 4 collections), and 13-25 × 6-10 (19 × 7) μ for pycnospores (90 spores from 9 collections).

Spore germination and viability. Diseased twigs bearing pycnospores of *Botryosphaeria ribis* were kept in the laboratory and spore germination tests were carried out at intervals. No quantitative observations were made but it was found that the spores germinated in water after 11 months, although there was a reduction in the germinating capacity which became apparent after 6 mo. An interesting feature in the germination of *Dothiorella* spores is the formation of septae which develop after the germ tubes have grown out. Two cross walls are generally formed, the septa developing about 5 hr. after germination has started.

CONCLUSION AND SUMMARY

From the experimental evidence presented above, it is possible to divide the evolution of *Botryosphaeria ribis* on tung trees into 4 phases, the first 3 of which are shown diagramatically in Figure 3:

(a) A period of latent infection, when the spores are dispersed by wind and lodge on the fruit surface.

(b) Penetration of the fungus into the fruit stalk about 1 to 2 months before fruit fall. Subsequent stem infection will depend upon the relative rates of development of the pathogen in the tissues and that of twig abscission.

(c) Twig blight, occurring early in the growing season when the new vegetative shoots arising below the fruit twigs are killed. In addition to *Botryosphaeria ribis*, this condition may also be brought about by *Botryodiplodia theobromae*.

(d) Dieback of large branches occurring either towards the end of the growing season or during following years when girdling of the stem has taken place. *Botryosphaeria ribis* alone appears to be responsible for this more severe phase of the disease.

The significance of this cycle in relation to control measures may now be discussed. It is clear that the first objective should be the early detection, thorough removal, and destruction of blighted twigs so as to prevent sporulation of the fungus. In order to be effective this operation must be done as soon as the trees burst into leaf. Infected branches must be cut well into healthy tissues to prevent further progress of the pathogen down the stem, and cut surfaces must be protected with fungicidal wound dressing (14). Protection by spraying or dusting may be necessary in heavily infected localities in view of the difficulties of destroying within a short time all the potential sources of infection. It must be remembered in this connection that *Botryosphaeria ribis* is not specific to *Aleurites* but occurs on many hosts which are widely cultivated. Although no experimental evidence is available on the time of the year when this operation should be carried out, a study of Fig. 3 will indicate that it is of primary importance that fruits (and probably apical buds) should be protected by a fungicidal coating as early as possible in the season, i.e., starting from about September. The southeast trade winds usually reach their maximum intensity in Nyasaland during September and October. This period of high wind is coincidental with both the early stages of sporulation and fruit development. Spore dispersal is probably more effective under these conditions than later in the season when the formation of fruiting bodies reaches a maximum. The main objects of fungicidal application are to protect the plant surface from infection, and to destroy the spores. From the observations recorded in this paper, spraying or dusting would be most effective between the months of September and December. Consequently, experiments have recently been laid out comparing the relative efficacy of copper and sulphur dusts applied in September, September and October, and September, October, and November.

DEPARTMENT OF AGRICULTURE
ZOMBA, NYASALAND
BRITISH CENTRAL AFRICA

LITERATURE CITED

1. BUGNICOURT, F. 1935. Contribution a l'etude du Sphaerostilbe repens B. and Br.; Bul. Econ. Indochine. **37**: 471-477.
2. DEPARTMENT OF AGRICULTURE, NYASALAND, Ann. Rep. 1940. (Abs. in Rev. Appl. Myc. **21**: 4.)
3. GALLOWAY, L. D. 1935. India, new plant diseases recorded in 1934. Int. Bul. Pl. Prot. **9**: 176-178.
4. GROSSENBACHER, J. G., AND B. N. DUGGAR. 1911. A contribution to the life history, parasitism and biology of Botryosphaeria ribis. N. Y. State Agr. Exp. Sta. Tech. Bul. **18**: 115-190.
5. LARGE, *J. R.* 1940. Parasitic diseases of tung. U. S. Dept. Agr. Pl. Dis. Rptr. **33**: 22-30.
6. LARGE, J. R. 1948. Canker of tung trees caused by Physalospora rhodina. Phytopathology **38**: 359-363.
7. MULLER, H. R. A. 1939. Aanteekeningen over eenige ziekten van Aleurites montana. Landbouw **15**: 54-68.
8. PLAKIDAS, A. G. 1936. Crown girdle of pear trees. (Abs.) Phytopathology **26**: 105.
9. PLAKIDAS, A. G. 1937. Diseases of tung trees in Louisiana. La. Agr. Exp. Sta. Bul. 282.
10. SHEAR, C. L., NEIL E. STEVENS AND MARGUERITE S. WILCOX. 1924. Botryosphaeria and Physalospora on

currant and apple. Jour. Agr. Res. (U. S.). **28**: 589-598.
11. Shear, C. L., Neil E. Stevens and Marguerite S. Wilcox. 1925. Botryosphaeria and Physalospora in the eastern United States. Mycologia **17**: 98-107.
12. Smith, Clayton O. 1934. Inoculations showing the wide host range of Botryosphaeria ribis. Jour. Agr. Res. (U. S.). **49**: 467-476.
13. Stevens, N. E., and C. L. Shear. 1929. Botryosphaeria and Physalospora in the Hawaiian Islands. Mycologia **21**: 313-320.
14. Webster, C. C., and P. O. Wiehe. 1950. Dieback of tung trees. Dept. Agr. Nyas. Pl. Dis. Circ. No. 1 (mimeographed).
15. Wiehe, P. O. 1950. Notes on three diseases of the tung tree in Webster, C.C., Cultivation of the tung oil tree in Nyasaland. Govt. Print. Zomba; pp. 37-41.

FACTORS AFFECTING THE SURVIVAL ABILITY OF CERTAIN PHYSIOLOGIC RACES OF PUCCINIA GRAMINIS AVENAE ERIKSS. & HENN.[1]

M. K. Hingorani [2]

Puccinia graminis avenae Eriksss. & Henn. has occasionally become destructively epidemic on oats over extensive areas in the United States. It comprises at least 13 physiologic races; a possible fourteenth race may be one collected in the United States on *Poa ampla* by Fischer and Claassen (3).

Annual surveys have shown that predominant races of *Puccinia graminis avenae* differ from year to year and from region to region. Levine and Smith (9) reported 5 races in the United States from 1921 to 1935, inclusive, with races 2 and 5 the most prevalent. Stakman and Loegering (14) recorded 6 races from more than 1200 isolates during the period 1939-1943, with races 2 and 5 comprising 90 per cent of the isolates. In 1943 and 1944, however, they found races 8 and 10 increasing to comprise over 30 per cent of all isolates and they recognized the possibility of a further increase in their prevalence. In 1946, races 8 and 10 comprised 54 per cent of the total number of isolates.

The reasons for this sudden increase of races 8 and 10 are not known. Newton and Johnson (11) suggest a physiologic change in these races such that they can now produce urediospores over a long period; another possible cause could be an increase in the acreage of susceptible varieties.

Although race 7 had occasionally been found near barberries, in the United States, it did not become prevalent until 1950. It had been postulated that if varieties such as Bonda, Mindo, Clinton, and others having White Tartar parentage should be extensively grown race 7 might develop in larger quantities, as did races 8 and 10, thus changing the situation with regard to resistant varieties.

[1] Accepted for publication May 16, 1952.
A condensation of a section of a thesis submitted to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy in 1947, while the author was a Fellow of the Watumull Foundation.
[2] The author wishes to acknowledge his indebtedness to Dr. E. C. Stakman, Chief of the Division of Plant Pathology, for counsel and advice in this work and to the Watumull Foundation for financial aid. He is also grateful to Dr. Helen Hart, Professor of Plant Pathology, for correcting the manuscript. At present the author is at the Indian Agricultural Research Institute, New Delhi, India.

Studies were therefore made on the effect of physiological and ecological factors on the relative survival ability of races 2, 7, and 8.

Materials and methods.—Races 2, 7, and 8 of oat stem rust were obtained from the Federal Rust Laboratory at University Farm, St. Paul, Minnesota. Only 1 isolate of each race was studied.

Cultures of these races were obtained by increasing the inoculum from a single well-isolated sorus of each race on a sparsely infected, highly susceptible host (Bond); periodic determinations of purity were made by inoculating the oat differentials, Jostrain (C.I. 1880), Richland (C.I. 787), and Minrus (C.I. 2144).

Classes and sub-classes of host reaction and infection types described by Stakman, Levine, and Bailey (12) were followed. The analytical key prepared by Newton and Johnson (10) was used for the identification of races. The techniques for collecting and inoculating are described in detail by Stakman, Levine, and Loegering (13).

Results of inoculating oats.—Since the principal host for the variety *avenae* is *Avena sativa* L., an attempt was made to see how far races 2, 7, and 8 were specific in their effects on different varieties of oats, and also to determine whether the 3 races differed sufficiently in host range as to affect their survival ability.

Fifty-nine varieties of oats, many of them obtained through the courtesy of T. H. Stanton of the United States Department of Agriculture, were inoculated with the 3 races. No variety was immune; but 2 hybrids, Hajira × Joanette and Victoria × (Hajira × Banner), were resistant to all 3 races. Thirty-one of the 59 varieties were susceptible to all 3 races: Alabama Red Rust-Proof C.I. 3934, Bond C.I. 2733, Carolina Red, Delta Red (88) C.I. 4220, Desota C.I. 3923, Ferguson C.I. 2150, Florilee C.I. 4060, Forkdeer C.I. 3170, Forvic C.I. 3846, Fulgrain (7), Fultex C.I. 3531, Fulton C.I. 3327, Gopher C.I. 2027, Kanota C.I. 839, Lega C.I. 3379, Lelina C.I. 3404, Leroy C.I. 3398, Letoria C.I. 3392, Nortex, New Nortex, Quincy Red C.I. 4077, Ranger C.I. 3417, Ruakura C.I. 2025, Rustler, Stanton (1) C.I. 3855, Stanton (3), Sterisel C.I. 2891, Travel-

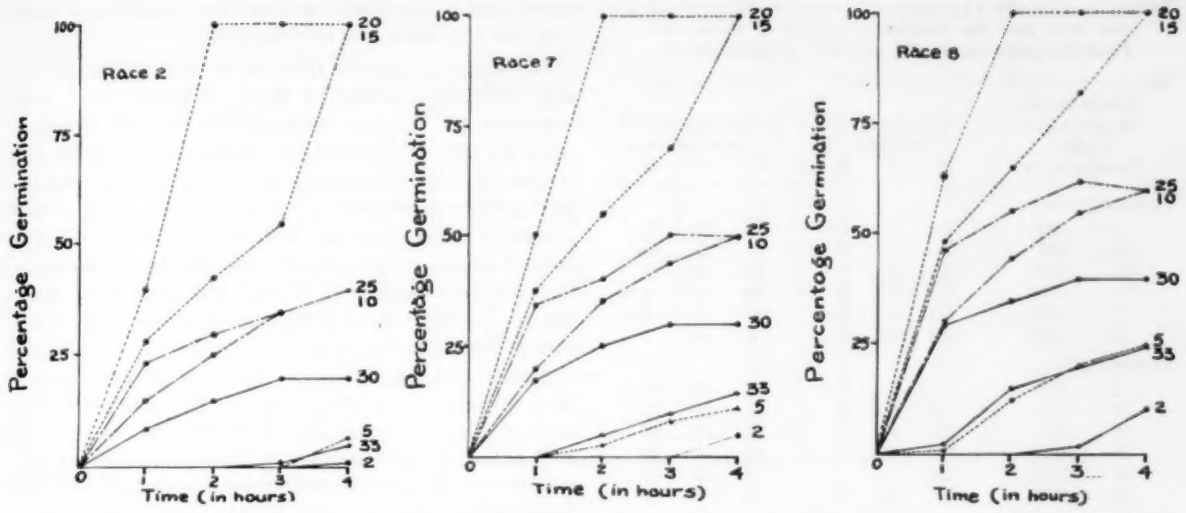


FIG. 1. The effect of temperature and time on the percentage germination of urediospores of races 2, 7, and 8 of *Puccinia graminis avenae*.

ler C.I. 4266, Victoria C.I. 2401, Victorgrain C.I. 4388, and Victory C.I. 1145.

Fifty of the 59 varieties were susceptible to race 8. In addition to the 31 varieties already mentioned there were 18 varieties susceptible to race 8 and resistant to races 2 and 7: Ajax C.I. 4157, Beaver C.I. 3837, Boone C.I. 3305, Exeter, Hajira C.I. 1001, Hancock, Iogold C.I. 2329, Lampton, Magistral, Neosho C.I. 4141, Nionark, Osage C.I. 3991, Otoe, Richland C.I. 787, Rohland, Tama C.I. 3502, Verde C.I. 4312, and Vicland C.I. 3611.

Only 38 of the 59 varieties were susceptible to race 7. Besides the 31 varieties that were susceptible to all 3 races, 7 varieties were susceptible to race 7 and resistant to races 2 and 8: Anthony C.I. 2143, Benton C.I. 3910, Bonda C.I. 4329, Clinton C.I. 3971, Green Mountain C.I. 1892, Mindo C.I. 4328, and Minrus C.I. 2144.

The 32 varieties that were susceptible to race 2 also had susceptibility to one or both of the other races. Thirty-one were susceptible to races 2, 7, and 8, while the variety Marion was susceptible to races 2 and 8 but was resistant to race 7.

Thus, race 8 apparently was more virulent than races 2 and 7 and had a wider host range in oats. It is obvious that susceptible varieties of oats can easily be a determining factor in the amount of rust inoculum produced by different races. Race 8 has, therefore, better chances of spread in grain-areas and as a result better chances of survival.

GERMINATION OF UREDIOSPORES.—Spores of approximately the same age and stage of maturity were used throughout these experiments. As soon as uredia were well formed, mature spores were collected by gently shaking the infected plants over a smooth funnel leading to a glass vial. The distilled water, slides, and Petri dishes to be used for germination tests had been placed at the particular germination temperatures at least 30 minutes before the spores were added to the water. Each percentage (Fig. 1) was based on 3 replicates of 500 spores at each temperature, counted after 1, 2, 3, and 4 hours.

The optimum temperature at which races 2, 7, and 8 germinate is the same. When the temperatures are beyond the optimum range, however, urediospores of race 8 germinate in greater number and faster than those of races 2 and 7. This is true both for low and high temperatures. Although many other factors are undoubtedly important, the results obtained indicate that race 8 should be able to establish and maintain itself under a wider range of temperature conditions, insofar as establishment and maintenance depend on germination of urediospores.

EFFECT OF TEMPERATURE AND LIGHT ON UREDIAL DEVELOPMENT.—The fact that environmental factors can induce striking changes in the reactions of differential hosts to physiologic races of oat stem rust has been demonstrated by several investigators (4, 5, 7, 11, 15). The writer attempted to study the infection types produced on Jostrain, Richland, and Minrus oats by the 3 races of rust and to determine the length of incubation period when these varieties were infected under different temperature and light conditions.

In one series of experiments greenhouse temperatures approximated 15°, 20°, and 30° C. and the light intensity varied from 300 to 6000 foot-candles. In another series in cold chambers the temperatures approximated 2°, 10°, and 20° C. and throughout the day and night the artificial light provided a light intensity of 200-300 foot-candles. The data from the 2 series have been combined in Table 1.

The incubation period was considered as beginning with inoculation of the oats seedlings and ending at the time that rust was fully developed. The shortest

TABLE 1.—*The effect of temperature and light on the infection type and the incubation period of three races of Puccinia graminis avenae on three varieties of oats*

Temperature (degrees C.): Light [a] (foot-candles)	Infection type for race			Incubation period in days for race		
	2	7	8	2	7	8
Jostrain variety						
2——300	X=	3	3+	70	60	55
10——300	X—	3+	4—	30	25	20
20——300	3+	4—	4	20	18	15
15——3000	X—	4—	4	10	9	8
20——3000	4+	4+	4+	6	5	5
30——3000	4+	4+	4+	6	6	5
Minrus variety						
2——300	1=	3	2—	72	60	60
10——300	1—	3+	2	33	25	22
20——300	2—	4—	2	25	18	16
15——3000	1+	4—	2—	12	9	10
20——3000	2+	4+	2	9	5	7
30——3000	2	4+	2	9	6	7
Richland variety						
2——300	0;	1—	3	72	65	55
10——300	1—	2—	3+	35	27	20
20——300	1	1+	3+	25	20	15
15——3000	1—	1	3+	12	10	7
20——3000	1+	1+	4+	9	7	5
30——3000	1	1+	4—	9	8	5

[a] Light ranged from 200 to 300 foot-candles or from 300 to 6000 foot-candles, but averages for the experiments approximated 300 and 3000 foot-candles.

incubation periods were observed at the high temperatures (20° and 30° C.) and the high light intensity (3000 foot-candles); but race 2 was always slower to develop than races 7 and 8. Reducing the light intensity to 300 foot-candles while maintaining the temperature at 20° C. generally trebled the length of the incubation period. Reducing both temperature and light intensity greatly prolonged the incubation period, but race 2 maintained its position of the slowest race even at 2° C. and 300 foot-candles of light.

Susceptibility of a variety to a race was almost as important as the environment in determining length of incubation period. The greater the susceptibility, the shorter the incubation period in a given environment. Thus, each race in a particular environment developed faster on a susceptible variety than on a resistant variety: race 2 always developed faster on Jostrain than on Minrus or Richland; race 7 developed faster on Jostrain and Minrus than on Richland; race 8 developed faster on Jostrain and Richland than on Minrus.

Nevertheless it must be assumed that inherent differences in the races themselves also influence length of incubation period. Race 8, as judged by incubation period, has better qualities for survival and spread than the other 2 races have. Race 8 consistently developed faster than race 7 and race 2 on 2 of the 3 varieties, Jostrain and Richland, in all the environments tested. On the third variety, Minrus, race 8 was somewhat slower than race 7 in the best environments, but it developed as fast as or faster than race 7 in the less favorable environments.

The highest infection types were observed at 20° C. and 3000 foot-candles of light, although in a few instances 30° C. was as favorable for rust development as 20° C. Departures from the optimum environment were responsible for slight changes in infection types produced by races 7 and 8. Race 2 was somewhat more variable with environment than the other 2 races: it was unable to sporulate on the resistant Richland oats at 2° C. and the susceptible reaction of Jostrain oats to it changed to a mesothetic reaction as temperature was reduced to 15° C. or lower.

Light Intensity.—Thirteen varieties of oats, grown in a greenhouse at 23°-25° C., were inoculated with each of the 3 rust races and duplicate pots were placed at 3 different light intensities. Plants exposed to full sunlight in the greenhouse received from 5000 to 9000 foot-candles, the average being 7000 foot-candles. Plants in a booth covered with 1 layer of cheesecloth received a medium light, from 500 to 1500, average of 1000, foot-candles. Those in a booth covered with a double layer of cheesecloth were in a low light intensity that averaged only 250 foot-candles but ranged from 100 to 400. Data on rust infection type and length of incubation period are in Table 2.

A reduced light intensity prolonged the incubation period but not to the same extent that had been observed on the differential varieties in the cold chambers at 20° C. and an artificial light of 300 foot-candles. On the 13 oat varieties 11-12 days with 250 foot-candles of light were sufficient for development of the slow race 2 as well as races 7 and 8.

Again the susceptibility of a variety to a race was an important factor in the rapidity of rust development. Race 8 had a slight advantage over race 7 and a greater advantage over race 2. Race 8 developed fastest on its 7 susceptible hosts, race 7 on its 7 susceptible hosts, and race 2 developed almost as fast as races 7 and 8 on Jostrain, the variety that was susceptible to all 3 races.

The infection types produced on these varieties were highest at the high light intensity but usually varied only slightly with less light. There were only 2 instances of qualitative changes: Marion oats became resistant to race 2 and Benton oats became moderately resistant to race 7 at the low light intensity.

It appears, therefore, that light, as a single environmental factor, may influence any race to some extent and some races to a considerable extent. The amount and kind of influence depend upon the race and the variety. It seems likely that race 8 may develop better than races 2 and 7 under reduced light. In nature, however, there usually is sufficient light so that it alone probably would not be a major factor in most grain-growing areas. Nevertheless, it can sometimes have considerable effect on rust development, especially in conjunction with temperature and probably with moisture.

TABLE 2.—*The effect of light on the infection type and the incubation period for races 2, 7, and 8 of Puccinia graminis avenae on 13 varieties of oats*

Variety	Infection type									Incubation period (days)								
	Race 2			Race 7			Race 8			Race 2			Race 7			Race 8		
	L	M	H[a]	L	M	H	L	M	H	L	M	H	L	M	H	L	M	H
Ajax	0;	1—	1	0;	1—	1	3	4—	4+	12	11	9	11	10	8	7	6	5
Anthony	1—	1+	2—	3—	3+	4	1	1+	2	12	10	9	8	8	7	10	9	8
Beaver	1—	1	1+	1—	1	1+	3	3+	4—	11	10	8	11	9	7	9	7	6
Benton	0;	1—	1	2—	3	4—	0;	1—	1	11	10	9	8	7	6	10	9	8
Bonda	0;	1	1+	3	3+	4	1	1+	2—	11	10	8	8	6	5	10	9	7
Clinton	0;	1—	1	3	3+	4	0;	1—	1+	12	11	9	8	7	6	11	9	8
Exeter	0;	1—	1+	0;	1—	1	3—	3	3+	11	9	8	10	8	7	8	6	5
Jostrain	3—	3+	4+	3	3+	4+	3	3+	4+	8	7	5	7	7	5	7	7	5
Mindo	0;	1—	1	3	3+	4	0;	1	1+	11	9	8	8	6	5	10	9	7
Marion	1+	3—	3+	0;	1—	1	3	3+	3+	11	9	8	10	8	7	7	6	6
Minrus	0;	1—	1	3+	3	4	1—	1	1+	11	10	8	7	6	5	10	8	6
Richland	0;	1	1+	1—	1	2—	3	3+	4+	12	11	9	11	10	9	7	6	5
Tama	0;	1	1+	1—	1	1	3	3+	4	12	11	9	11	9	9	7	6	5

[a] Low light intensity (L) averaged 250 foot-candles; medium light intensity (M) averaged 1000 foot-candles; and high light intensity (H) averaged 7000 foot-candles.

TELIAL FORMATION AND FACTORS AFFECTING IT.—It is obvious that races which develop telia rapidly in nature may be greatly handicapped owing to curtailment in their uredial production (1, 5). It appears that such races may not become so rapidly distributed during summer. Telial formation in races 2, 7, and 8 was therefore studied to see whether they differed in their readiness to form telia and if there was a correlation between rarity of race 7 in nature and its telial development. The effect of temperature and light on telial formation was also determined.

The varieties Bond and Jostrain were used because they were equally susceptible to the 3 races. The temperatures maintained were 30°, 20°, 15°, and 2°C. The first 3 temperatures were obtained in the greenhouse with natural light; 2° C. was maintained in a cold chamber where artificial light (200-300 foot-candles) was supplied continuously. In another experiment, low, medium, and high light intensities averaged about 250, 1000, and 7000 foot-candles, respectively, and temperature approximated 23°-25° C. The results on Bond oats are summarized in Tables 3 and 4. Results with Jostrain were very similar to those for Bond.

TABLE 3.—*Time required for telial formation by races 2, 7, and 8 of Puccinia graminis avenae on Bond oats at 4 different temperatures*

Race and Temperature	Number of days from inoculation to First telial formation		Complete telial formation	
	Seedlings	Adults	Seedlings	Adults
Race 7				
30°C.	12	13	16	19
20°C.	15	17	20	24
15°C.	30	32	39	41
2°C.	54	55	60	62
Race 2[a]				
2°C.	60	65	70	80
Race 8[a]				
2°C.	65	65	85	87

[a] Races 2 and 8 did not form telia at 15°, 20°, or 30°C.

Race 7 formed telia rapidly and abundantly on susceptible hosts both in the seedling and adult stages. Telia formed more rapidly at high temperatures than at low ones and somewhat more rapidly at high light intensities than at lower intensities. The author also has observed that race 7 forms telia rapidly on grasses.

Races 2 and 8, on the other hand, failed to form telia under conditions that favored development of uredia; nevertheless they produced telia at the unfavorable temperature of 2° C.

Thus race 7, which is only occasionally found near barberries, forms telia readily; whereas races 2 and 8, which are more commonly found in nature, apparently do not produce them so readily. These studies seem to support the contention of Gordon (5) that the races that produce telia more rapidly are the ones found more rarely in nature.

GERMINATION OF TELIOSPORES.—Teliospores of *Puccinia graminis* ordinarily require a resting period before germination. This is usually about 6 months in Minnesota for telia collected in late summer or early fall. Cotter (2) showed, however, that there were racial differences in *Puccinia graminis tritici* with respect to telial germination.

To shorten the rest period, greenhouse-formed teliospores of the 3 races of oat stem rust were frozen at

TABLE 4.—*Time required for telial formation by race 7[a] of Puccinia graminis avenae on Bond oats under different light intensities and at 23°-24° C.*

Light intensity (foot-candles)	Number of days from inoculation to First telial formation		Complete telial formation	
	Seedlings	Adults	Seedlings	Adults
High: 7000	15	15	25	25
Medium: 1000	17	15	25	25
Low: 250	21	22	26	28

[a] Races 2 and 8 did not form telia.

—5° C. for 7 days then thawed and sprayed with cold tap water for 7 days, as suggested by Johnson and Newton (8). This preliminary freezing and thawing was followed by alternate drying and wetting treatments, each of 2 days' duration. After the fourth drying period and each successive one through the tenth the telispores of race 7 germinated, but these treatments were not successful with races 2 and 8. Barberries were inoculated with the teliospores of race 7 and became infected. Apparently the teliospores of race 7 do not require the usual 6-month rest period.

Several environmental factors were varied in testing races 2 and 8: constant temperatures of 10°, 15°, and 25° C. were provided during the germination period; teliospores were treated with various chemicals such as benzaldehyde, butyric acid, and citric acid, in order to increase the permeability of spore walls to water and oxygen. In no case did teliospores of races 2 and 8 germinate.

Selfing studies with race 7.—The earliest record of an artificial selfing of a known race of oat stem rust is that of race 8 reported by Gordon and Welsh (6). In a study of cultures arising from 16 separate aecial cups and 4 mass transfers of aecia, race 8 occurred 15 times, race 6 seven times, and race 7 only once. Johnson and Newton (8) found that races 2, 7, and 10a were homogeneous and that races 3, 5, and 8 were heterogeneous with respect to their infection types on one or the other of the 3 differentials used. From race 8 they obtained races 6 and 8.

The procedure adopted in the selfing studies reported herein has been described in detail by Newton and Johnson (10). To gain an idea of the numerical distribution of physiologic races in the progeny of selfing, single aecial cups were picked at random from numerous aecial pustules, crushed, and transferred individually to seedling leaves of Bond oats. One hundred such isolations were made, of which 95 were successful.

The results show that race 7 is relatively homogeneous with respect to factors for pathogenicity. From the isolations made, only race 7 was obtained. The infection type produced on Minrus and Jostrain oats ranged from 4— to 4. On Richland, however, the infection types varied from 0; to 2. Infection type 0; was produced by 25 isolates, types 0; to 1 by 40 isolates, type 1 by 8 isolates, type 0; to 2 by 2 isolates, types 1 to 2 by 16 isolates, and type 2 by 4 isolates.

Thus it is clear that recombinations have occurred on barberries which, although they do not transcend the infection types normally produced by race 7 on the 3 differentials, result in some quantitative changes on Richland. This strongly suggests that there are probably several biotypes of race 7 which differ quantitatively from one another in their virulence, at least on Richland oats.

Discussion.—During the past few years, race 8 of *Puccinia graminis avenae* has increased greatly in prevalence in the United States with a corresponding decrease in race 2. The reasons for this shift have not been properly explained. The present findings help to clarify certain points regarding this question.

The writer has been able to study the effects of only a limited number of factors under a limited range of environmental conditions. The facts obtained, however, do indicate that races 2, 7, and 8 of *Puccinia graminis avenae* differ somewhat in their physiological characters. Some of the factors are valuable in the struggle for existence; others are unfavorable.

Race 8, insofar as the writer's data are indicative, seems to have more valuable characteristics than races 2 and 7 and no conspicuous weaknesses. It is unlikely, however, that the increase in prevalence and distribution of race 8 in recent years has been due to a combination of environmental conditions that are especially favorable to it. As long as susceptible hosts are available, therefore, this race can be expected to retain at least its present position of importance. Race 2 has really no conspicuous weaknesses, but will of course tend to decrease as the acreage of resistant varieties increases.

Data regarding race 7 are somewhat conflicting. The tendency to produce telia quickly may handicap it, especially in non-barberry areas. On the other hand, observations on the rapidity with which race 7 spread in the experimental plots at the Minnesota Agricultural Experiment Station in the summer of 1947 suggest that it may have certain characteristics that compensate for its one conspicuous weakness. Survival value may not necessarily be indicated by the algebraic sum of favorable and unfavorable characteristics, if they could be given mathematical values, because of the very important elements of sequence of events in time.

SUMMARY

Race 8 of *Puccinia graminis avenae* was relatively more virulent than races 2 and 7 on oats.

Urediospores of race 8 germinated better and faster than those of races 2 and 7.

Temperature and light affected the incubation period and the uredial development of the 3 races. Race 2 was the most sensitive in this respect.

Race 7 produced telia rapidly under all conditions, whereas races 2 and 8 formed them only at 2° C. Temperature and light affected telial formation in race 7.

Teliospores of race 7 did not require the usual rest period, but those of races 2 and 8 do.

Race 7 was found to be relatively homogeneous with respect to factors for pathogenicity on the 3 differential hosts. However, it comprised several biotypes which differed in their effects on Richland oats.

University Farm
St. Paul, Minnesota

LITERATURE CITED

1. Bailey, D. L. 1925. Physiologic specialization in Puccinia graminis avenae Erikss. and Henn. Minn. Agr. Exp. Sta. Tech. Bul. 35.

2. COTTER, R. U. 1940. Unseasonable germination of teliospores of Puccinia graminis tritici. Phytopathology **30:** 689-691.
3. FISCHER, G. W. AND C. E. CLAASSEN. 1944. Studies of stem rust (Puccinia graminis) from Poa ampla, Avena fatua, and Agropyron spicatum in the Pullman, Washington region. Phytopathology **34:** 301-314.
4. GORDON, W. L. 1930. Effect of temperature on host reactions to physiologic forms of Puccinia graminis avenae Erikss. and Henn. Scient. Agr. **11:** 95-103.
5. GORDON, W. L. 1933. A study of the relation of environment to the development of the uredinial and telial stages of the physiologic forms of Puccinia graminis avenae Erikss. and Henn. Scient. Agr. **14:** 184-237.
6. GORDON, W. L. AND J. N. WELSH. 1932. Oat stem rust investigation in Canada. Scient. Agr. **13:** 228-235.
7. HINGORANI, M. K. 1951. The effect of temperature and light on the mesothetic reaction of Jostrain oats to stem rust. Indian Phytopathology **4:** 85-87.
8. JOHNSON, T. AND M. NEWTON. 1932. Crossing and selfing studies with physiologic races of oat stem rust. Canad. Jour. Res. (C) **18:** 54-67.
9. LEVINE, M. N. AND D. C. SMITH. 1937. Comparative reaction of oat varieties in the seedling and maturing stages to physiologic races of Puccinia graminis avenae, and the distribution of these races in the United States. Jour. Agr. Res. [U. S.] **55:** 713-729.
10. NEWTON, M. AND T. JOHNSON. 1932. Studies in cereal diseases. VIII. Specialization and hybridization of wheat stem rust, Puccinia graminis tritici, in Canada. Canada Dept. Agr. Bul. 160.
11. NEWTON, M. AND T. JOHNSON. 1944. Physiologic specialization of oat stem rust in Canada. Canad. Jour. Res. (C) **22:** 201-216.
12. STAKMAN, E. C., M. N. LEVINE, AND D. L. BAILEY. 1923. Biologic forms of Puccinia graminis on varieties of Avenae spp. Jour. Agr. Res. [U. S.] **24:** 1013-1018.
13. STAKMAN, E. C., M. N. LEVINE, AND W. Q. LOEGERING. 1944. Identification of physiologic races of Puccinia graminis tritici. U. S. Dept. Agr. B.E.P.Q. Sect. E. 617.
14. STAKMAN, E. C. AND W. Q. LOEGERING. 1944. The potential importance of race 8 of Puccinia graminis avenae in the United States. Phytopathology **34:** 421-425.
15. WATERHOUSE, W. L. 1929. Australian rust studies. I. Proc. Linn. Soc. N. S. W. **55:** 159-178.

A PHYTOPATHOLOGICAL NOTE

Mycelial mats of Chalara quercina on killed oak trees as a potential source of perithecia in nature.[1] H. L. BARNETT, JOHN M. STALEY, AND R. P. TRUE. The method of long distance spread of oak wilt and the agents involved in the dissemination of spores are among the major unsolved phases of the oak wilt problem. The importance of the ascospores in the spread of the fungus is still unknown. Ascospores have been used successfully in artificial inoculation trials[2] and we may assume that both conidia and ascospores may be responsible for natural infections. Prior to this time there have been only a few reports of perithecia in nature[3] but it is reasonable to assume that their occurrence is much more common than previously suspected.

During 1951 mycelial mats of *Chalara quercina* producing abundant conidia were discovered in Illinois on standing oaks killed by the fungus and on felled oak wood on the forest floor.[4] Similar mycelial mats were found in West Virginia during the latter part of April, 1952, on standing and felled oaks killed during 1951. They were usually gray in color, elliptical in shape, extending to a length of several inches, and were usually attached either to the inner surface of the bark or to the outer surface of the wood. Some mycelial mats of limited extent were found at the cork cambium layer. Pure cultures of *C. quercina* were easily obtained from the conidia of these mats, although other fungi were often present.

The presence of the mats on dead oak wilt trees and the fact that the bark was usually cracked, permitting the entrance of insects, raised the question as to whether the mats might be capable of producing perithecia under proper conditions. Microscopic examination revealed an abundance of conidia and on some mats the presence of sterile perithecial structures like those often formed on single spore cultures in the laboratory.

Entire freshly collected mats or portions of them were placed in moist chambers at 25° C. and water suspensions of conidia from pure cultures were used to spermatize certain areas, using the technique of Hepting et al.[5] Loopfuls of the spore suspension were used in the first trials, but later an atomizer was used. In some trials conidia from single spore cultures were used, while in others conidia of both compatibility groups were mixed. A total of 12 mycelial mats from 3 trees, each from a different county in West Virginia have been used. When the conidia from the proper compatibility group were used in the spermatization process, all the freshly collected mycelial mats tested produced fertile perithecia and ascopores within 3 days (Fig. 1). Because of the thickness of the mycelial mat, the perithecia sometimes appear almost

[1] Published with the approval of the director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 457.

[2] Bretz, T. W. 1952. The perithecial stage of *Chalara quercina* Henry. (Abs.) Phytopathology 42: 3.

[3] Perithecia were found in Illinois in 1951, according to personal correspondence with J. C. Carter and L. R. Tehon. No other reports of their occurrence in nature are known to the authors.

[4] Curl, E. A., G. J. Stessel and B. M. Zuckerman. 1952. Macroscopic growth of the oak wilt fungus in nature. (Abs.) Phytopathology 42: 6.

[5] Hepting, G. H., E. R. Toole, and J. S. Boyce, Jr. 1952. Sex and compatibility in the oak wilt fungus. Plant Dis. Reptr. 36: 64.

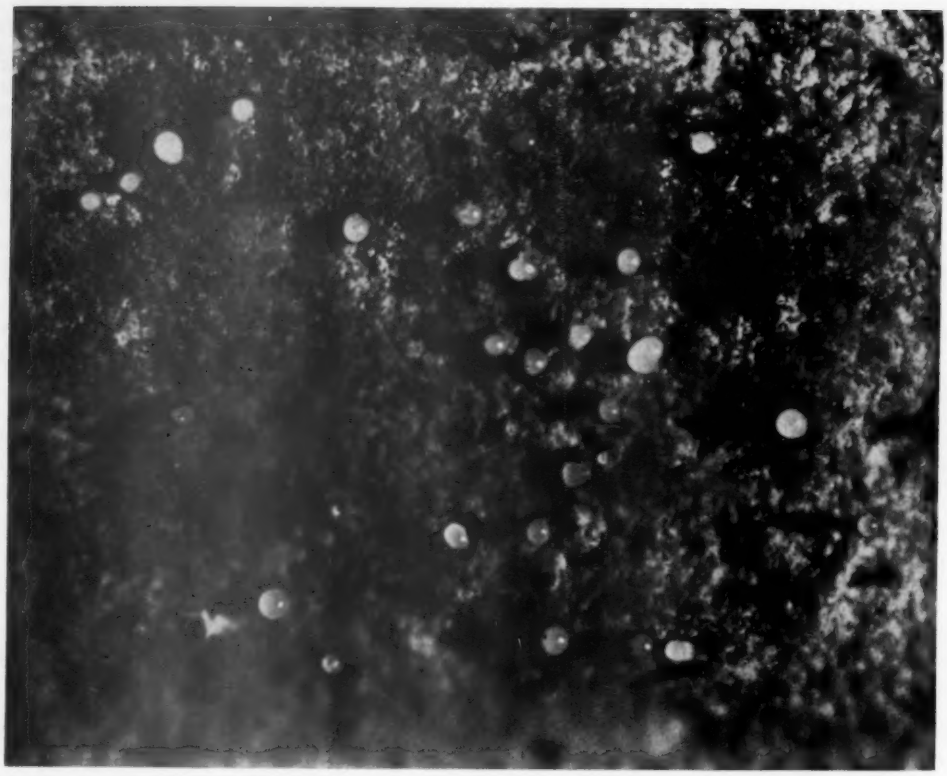


FIG. 1. Perithecia of *C. quercina* with droplets of ascospores on mycelial mats collected in nature and spermatized with conidia in the laboratory (×18).

beakless, and the mass of ascospores only slightly raised above the surface.

On May 3, 1952, mycelial mats were collected from trees in southern and western West Virginia and brought directly in the laboratory. Close examination revealed the presence of fertile perithecia producing typical ascospores on mycelial mats from 2 trees in different counties. This finding has supplied the needed proof that the mycelial mats are capable of producing perithecia under the right conditions in nature as well as in the laboratory.

The important fact is that the mycelial mats occurring beneath the loose cracked bark are ready for spermatization and are capable of producing perithecia quickly when conidia of the opposite compatibility group are introduced. The significance of this seems clear. Insects, attracted to these mycelial mats that emit a characteristic odor, may visit several trees within a local area, carrying conidia from one mycelial mat to another. If both compatibility groups are present within this area the act of spermatization may thus be accomplished and perithecia may develop. Insects visiting these mats at this time could serve as vectors of both conidia and ascospores.—Department of Plant Pathology and Bacteriology, West Virginia University, Morgantown, West Virginia.

SIX NEW LEAFHOPPER VECTORS OF PIERCE'S DISEASE VIRUS[1]

J. H. Freitag, N. W. Frazier and R. A. Flock

Hewitt, Frazier and Houston (6) first demonstrated the transmission of Pierce's disease virus by leafhoppers of the 2 genera *Draeculacephala* and *Carneocephala*. This work was confirmed by Hewitt, Frazier, Jacob and Freitag (5) who stated that 3 species of leafhoppers, the green sharpshooter, *Draeculacephala minerva* Ball, the red-headed sharpshooter, *Carneocephala fulgida* Nottingham and the blue-green sharpshooter, *Hordnia* (= *Cicadella* auct.) *circellata* (Baker) are vectors of the virus. These 3 species are widely distributed in California and are considered to be the economically important vectors in the spread of the virus in vineyards and alfalfa fields. Frazier (3) reported 6 additional vectors, *Carneocephala triguttata* Nottingham, *Helochara delta* Oman, *Neokolla severini* DeLong (as *gothica* Signoret), *Keonolla* (= *Neokolla* auct.) *confluens* (Uhler) and *Cuerna occidentalis* Oman and Beamer. He pointed out the phylogenetic relationship existing among the then known vectors, all of which were contained in the subfamily Tettigellinae, and stated that any species of the subfamily should be considered a potential vector.

Frazier and Feitag (4) demonstrated that 5 additional species of leafhoppers were vectors of the virus and these included *Pagaronia triunata* Ball, *P. 13-punctata* Ball, *P. furcata* Oman, *P. confusa* Oman and *Friscanus friscanus* (Ball).

Severin (14) reported 4 species of spittle insects (Cercopidae), *Aphrophora angulata* Ball, *A. permutata* Uhler, *Clastoptera brunnea* Ball and *Philaenus leucophthalmus* (Linnaeus) to be vectors of Pierce's disease virus.

The present investigation reports results of recent tests to determine if other California leafhopper species of the subfamily Tettigellinae were capable of transmitting Pierce's disease virus.

METHODS AND MATERIALS.—Experimental methods were similar to those reported in a previous paper (4). Leafhoppers were collected in the field and were usually fed for 1 or 2 days on experimentally infected grape and alfalfa plants. They were then transferred in lots of 2 to 20 leafhoppers to healthy grape and alfalfa plants for a period of from 2 to 10 days, usually 7. The inoculated test plants were kept under observation for 6 months to more than a year.

RESULTS.—Results in Table 1 indicate that the 9 species of leafhoppers tested proved capable of transmitting the virus. Six species are new leafhopper vectors. These are *Draeculacephala californica* Davidson and Frazier, *D. noveboracensis* (Fitch), *D. crassicornis* Van Duzee, *Graphocephala cythura* (Baker), *Homalodisca liturata* Ball, and *Cuerna yuccae* Oman *and Beamer*. Three species, *Cuerna occidentalis* Oman and Beamer, *Keonolla confluens* (Uhler) and *Neokolla hieroglyphica* (Say) have been previously reported as vectors (4). Additional tests were made with these latter 3 species since the previous report was based on low numbers of tests and because large field populations were available for testing.

TABLE 1.—*Leafhopper transmission of the Pierce's disease virus from diseased grape and alfalfa to healthy grape and alfalfa plants*

Species of leafhopper	From diseased grape		From diseased alfalfa		Percentage of total infected
	To grape	To alfalfa	To grape	To alfalfa	
Draeculacephala californica D. & F.	1/1[a]	1/1	1/3	5/9	57
D. noveboracensis (Fitch)	6/16	1/10	3/12	0/13	20
D. crassicornis Van Duzee	12/13	5/11	7/16	8/17	56
Graphocephala cythura (Baker)	5/14	1/14	1/10	2/7	26
Homalodisca liturata Ball	3/3	3/3	5/7	0/5	61
Cuerna yucccae Om. & B.	4/5	4/5	2/16	0/14	25
C. occidentalis Om. & B.[b]	33/37	2/12	7/9	1/10	63
Keonolla confluens (Uhler)[b]	4/11	1/5	0/1	1/2	32
Neokolla hieroglyphica (Say)[b]	7/31	0/26	3/24	1/23	11

[a] Numerator indicates number of plants infected; denominator indicates number of plants inoculated.
[b] Previously reported vectors (4).

The 4 species which proved to be the most efficient vectors were *Cuerna occidentalis, Homalodisca liturata, Draeculacephala californica* and *D. crassicornis*, all of which infected more than 50 per cent of the test plants. The variation in the ability of the leafhoppers to acquire virus from grape and alfalfa and their ability to infect these 2 host plants was apparently related to the capacity of the insects to survive on the 2 plants. The results indicate that grape was both a better virus source and test plant.

DISCUSSION.—Only aster yellows virus has been transmitted by more species of leafhoppers than has Pierce's disease virus. Severin (8, 9, 10, 11, 12, 13) has demonstrated California aster yellows virus to be experimentally transmitted by 23 species of leafhoppers.

Results of this work and of previous studies have now demonstrated that 20 species of leafhoppers (4, 5, 6) and 4 species of spittle insects (14) are capable of transmitting Pierce's disease virus. The 20 species

[1] Accepted for publication May 24, 1952.

of leafhoppers all belong to the subfamily Tettigellinae and every species of this group that has been tested has proved a vector of the virus. All species of this subfamily are under suspicion as potential vectors of the virus.

Members of this group of leafhoppers which occur in California are found in a great variety of habitats and have very diverse host plants. Some species are limited to a very few hosts while others have a wide range of food and breeding plants. The great majority of them have not commonly been observed to occur in vineyards or alfalfa fields and consequently are not considered important in the dissemination of the virus to its economic host plants. Only *Draeculacephala minerva, Carneocephala fulgida* and *Hordnia circellata* have been found to occur abundantly in vineyards and alfalfa fields. All the vectors may be of some importance on wild host plants which might serve as natural virus reservoirs.

It has been commonly observed that leafhopper vectors of Pierce's disease virus excrete large quantities of liquid when fed on plants. This fact suggested that the insects were feeding in the vascular tissues of the plant where they might acquire the large amounts of water which they excrete. Houston, Esau and Hewitt (7) have demonstrated that the economically important vectors of the virus feed in the xylem tissue of the grape and alfalfa plant.

Esau (2) has further shown that anatomic changes in affected plants, considered with external symptoms and mode of insect transmission of Pierce's disease virus, suggest a close association of the virus with the xylem tissue of the plant. She has indicated that the phony peach virus, like Pierce's disease virus, induces gum development in the xylem and that these 2 viruses are similar in the internal symptoms they produce in infected plants.

Since phony peach virus and Pierce's disease virus are similar in their host tissue relationships it is not surprising that they should be transmitted by closely related vectors. Turner (15) reports 4 species of the subfamily *Tettigellinae, Cuerna costalis* (Fabricius), *Graphocephala versuta* (Say), *Homalodisca triquetra* (Fabricius) and *Oncometopia undata* (Fabricius), as being vectors of the phony peach virus. It is interesting that species of the first 3 genera reported by Turner have also been shown to be vectors of Pierce's disease virus.

A third virus which may involve infection of xylem tissue has been shown to be transmitted by a leafhopper of the Tettigellinae. Abbot and Ingram (1) demonstrated *Draeculacephala portola* Ball to be a vector of chlorotic streak virus of sugar cane.

SUMMARY

Six additional leafhopper vectors of the Pierce's disease virus were demonstrated, by transmission of the virus from infected grape and alfalfa plants to previously healthy grape and alfalfa test plants. The leafhoppers all belong to the subfamily Tettigellinae and include the following species: *Draeculacephala californica* Davidson and Frazier, *D. noveboracensis* (Fitch), *D. crassicornis* Van Duzee, *Cuerna yuccae* Oman and Beamer, *Graphocephala cythura* (Baker) and *Homalodisca liturata* Ball. Additional experimental transmission of the virus by 3 previously reported leafhoppers *Cuerna occidentalis* Oman and Beamer, *Neokolla hieroglyphica* (Say) and *Keonolla confluens* (Uhler) is presented.

The present work brings to a total of 20 the list of species of leafhoppers that have been shown to be vectors of Pierce's disease and includes the common species of the subfamily in all parts of the state.

Since the leafhoppers discussed in this paper have not been commonly observed in vineyards and alfalfa fields, they are not considered to be important in the dissemination of the virus to its economic host plants. They may be involved in the spread of virus to wild host plants which constitute a natural virus reservoir.

Division of Entomology and Parasitology
College of Agriculture, University of California
Berkeley and Riverside, California

LITERATURE CITED

1. Abbott, E. V. and J. W. Ingram. 1942. Transmission of chlorotic streak of sugar cane by the leafhopper Draeculacephala portola. Phytopathology **32:** 99-101.
2. Esau, Katherine. 1948. Anatomic effects of the viruses of Pierce's disease and phony peach. Hilgardia **18:** 423-482.
3. Frazier, N. W. 1944. Phylogenic relationship of the nine known leafhopper vectors of Pierce's disease of grape. (Abs.) Phytopathology **34:** 1000.
4. Frazier, N. W. and J. H. Freitag. 1946. Ten additional leafhopper vectors of the virus causing Pierce's disease of grapes. Phytopathology **36:** 634-637.
5. Hewitt, W. B., N. W. Frazier, H. E. Jacob and J. H. Freitag. 1942. Pierce's disease of grapevines. Cal. Agr. Exp. Sta. Circ. 353.
6. Hewitt, W. B., N. W. Frazier and B. R. Houston. 1942. Transmission of Pierce's disease of grapevines with a leafhopper. (Abs.) Phytopathology **32:** 8.
7. Houston, B. R., Katherine Esau and W. B. Hewitt. 1947. The mode of vector feeding and the tissues involved in the transmission of Pierce's disease virus in grape and alfalfa. Phytopathology **37:** 247-253.
8. Severin, H. H. P. 1945. Evidence of nonspecific transmission of California aster-yellow virus by leafhoppers. Hilgardia **17:** 23-59.
9. Severin, H. H. P. 1946. Transmission of California aster-yellows virus by the first reported leafhopper vector in Gyponinae. Hilgardia **17:** 141-153.
10. Severin, H. H. P. 1947. Acinopterus angulatus, a newly discovered vector of California aster-yellows virus. Hilgardia **17:** 197-205.
11. Severin, H. H. P. 1947. Newly discovered leafhopper vectors of California aster-yellows virus. Hilgardia **17:** 511-519.
12. Severin, H. H. P. 1948. Transmission of California aster-yellows virus by leafhopper species in Thamnotettix group. Hilgardia **18:** 203-216.
13. Severin, H. H. P. 1950. Texananus incurvatus II. Transmission of California aster-yellows virus. Hilgardia **19:** 544-545.
14. Severin, H. H. P. 1950. Spittle-insect vectors of Pierce's disease virus. II. Life history and virus transmission. Hilgardia **19:** 357-382.
15. Turner, W. F. 1949. Insect vectors of phony peach disease. Science **109:** 87-88.

VEIN CLEARING ON SUGAR BEET INDUCED BY THE BEET LEAFHOPPER [1]

C. W. Bennett

On several occasions since 1930 a type of vein clearing similar to that produced by the curly-top virus was noted on young leaves of small sugar beet plants on which the beet leafhopper, *Circulifer tenellus* (Bak.), was feeding. At first it was suspected that this vein clearing was caused by an attenuated strain of the curly-top virus. However, it was soon found that young leaves produced after the leafhoppers were removed from the plant did not show vein clearing. Recovery of this type has not been observed in plants infected with curly top. Furthermore, numerous attempts to transmit virus from such plants by means of the beet leafhopper failed to indicate the presence of an infectious agent. It was concluded, therefore, on the basis of this and other evidence that this type of vein clearing is not produced by a virus but by a toxin introduced by the insects in feeding.

The percentage of insects from stock cultures or from field collections that possess the ability to induce vein clearing is very low. Rarely has it exceeded 1 insect in 200 and in numerous instances caging of large numbers of insects singly on seedling plants has resulted in no injury. The development of a strain of the beet leafhopper with a high incidence of ability to produce vein clearing has made possible a more detailed study of the injury and factors involved in its production.

RESULTS OF SELECTION AND MATING LEAFHOPPERS WITH ABILITY TO PRODUCE VEIN CLEARING.—In 1939, 600 leafhopper nymphs from nonviruliferous stock colonies were caged singly on seedling sugar beet plants; 3 plants showed marked vein clearing. The 3 leafhoppers, 1 male and 2 females, from these plants were placed in a breeding cage and allowed to reproduce. No attempt was made to record generations of insects, but after a large colony of progeny was available, nymphs were selected and caged singly on seedling sugar beet plants. Those from plants on which the most severe vein clearing occurred were selected and placed in a breeding cage. This process of selection and random mating was continued through 5 more selections at intervals over a period of about 2 years.

Results of tests of the progenies from the different selections are in Table 1. It is evident that ability of the leafhoppers to induce vein clearing was markedly increased by selection. Tests of individuals from the colony of the last matings showed that nearly 60 per cent of the leafhoppers produced vein clearing. Progeny from selection 6 were kept as colonies of nonviruliferous leafhoppers without further selection from 1942 to 1950 and then tested. Well over 50 per cent of the individuals were able to induce vein clearing when caged singly on seedling sugar beet plants.

INJURY INDUCED BY LEAFHOPPERS OF THE SELECTED STRAIN.—*Macroscopic Effect.*—Injury induced by feeding of insects of the selected strain was more severe than that caused by feeding of insects from stock colonies or field collections. Usually vein clearing appeared on young leaves 2 to 4 days after leafhoppers began feeding. It was more pronounced on, and often restricted to leaves on the side of the plant on which the leafhopper was caged. Usually all the veins of an affected area showed injury. Cleared veins were visible even by reflected light and showed a marked resemblance to cleared veins caused by some strains of curly-top virus. However, the cleared areas along the veins were broader and more uniform in width and the lines were not interrupted to form short lines or dots as is sometimes true with curly top. In mild cases the leaves were not distorted nor appreciably retarded in growth. More severely affected leaves showed dwarfing and various types of distortion in addition to extensive vein clearing as shown in Figure 1. In some instances growth was more retarded along the margin of the leaves than in the midrib region, resulting in a blade resembling an inverted canoe. Sometimes growth was more retarded on one side, causing the leaf to curve laterally. No vein swelling nor distortion of the type produced by curly top was observed. As with curly top, the vein clearing became less marked or disappeared as the leaf matured.

Cytological Effect.—Judging from the lack of vein swelling and other abnormalities, it seemed probable that the toxic agent introduced by the beet leafhopper caused only minor cytological and anatomical disturbances. Cleared veins killed, sectioned, and stained by a standard cytological technique, appeared indistinguishable from cross sections of normal veins. Also, free-hand sections of affected veins were made and compared with similar sections from normal leaves. In all such sections, shape, size, arrangement, and number of cells of the different tissues appeared to be unaffected by the vein clearing. However, in the fresh sections chloroplasts in the parenchyma cells immedi-

TABLE 1.—*Results of tests of progenies of successive selections of the beet leafhopper for ability to induce vein clearing in sugar beet plants*

Selection number	Number of leafhoppers tested	Number of leafhoppers that induced vein clearing	Percentage of tested leafhoppers that induced vein clearing
2	360	17	4.7
3	320	48	15.0
4	311	165	53.1
5	220	128	58.0
6	267	159	59.6
Check [a]	100	0	0.0

[a] Stock nonselected leafhopper tested at the same time as leafhoppers of selection 6.

[1] Accepted for publication June 13, 1952.

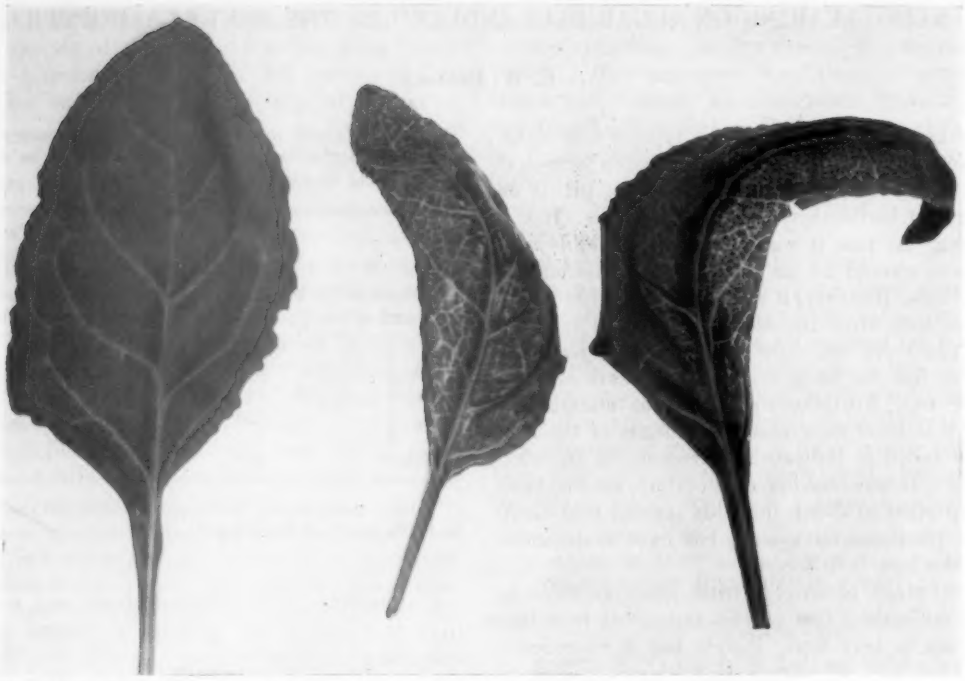


FIG. 1. Normal leaf of sugar beet (left) with 2 leaves (middle and right) showing vein clearing, dwarfing, and cupping induced by toxin introduced by individuals of a selected strain of the beet leafhopper.

ately surrounding the vascular bundles were paler and some plastids seemed to be devoid of green color. Thus it appears that vein clearing of this type is due to the temporary or permanent partial suppression of chlorophyl formation in parenchyma cells close to the vascular bundles. The distribution of the toxin from the point of introduction parallels closely the path of distribution of curly-top virus in plants of the same size. Thus, movement and distribution appear to be closely correlated with movement and distribution of elaborated food materials, and probably occur mainly in the phloem.

INFLUENCE OF SIZE AND TYPE OF PLANT AND AMOUNT OF TOXIN ON PRODUCTION OF VEIN CLEARING.—Tests were made in which different numbers of leafhoppers were caged on rapidly growing sugar beet plants to determine the influence of different amounts of toxin introduced into the plants. In a typical test, 240 plants with 6 to 8 true leaves were divided into 6 lots of 40 plants each. Leafhoppers were caged on leaves of each plant of the respective lots as follows: Lot 1, 1 leafhopper on 1 of the larger leaves; Lot 2, 2 leafhoppers singly on opposite leaves; Lot 3, 5 leafhoppers in 1 cage on 1 leaf; Lot 4, 5 leafhoppers in each of 2 cages on opposite leaves; Lot 5, 10 leafhoppers in 1 cage on 1 leaf; Lot 6, 10 leafhoppers in each of 2 cages on opposite leaves.

Vein clearing was observed on 24, 29, 31, 30, 27, and 29 plants, respectively, of Lots 1 to 6, inclusive. There were no obvious differences in the severity of injury on plants of the different lots, but the plants with leafhoppers caged on 2 leaves on opposite sides showed a more extensive distribution of injury. Results indicate that a single leafhopper possesses the ability to induce vein clearing and is able to introduce enough toxin into a plant of the size used in this test to produce about a maximum of vein clearing. The fact that the percentage of plants showing vein clearing did not vary greatly in the different lots indicates that, under the conditions of the test, only about $\frac{2}{3}$ of the plants were susceptible to injury by introduced toxin; had all plants been susceptible to injury, higher percentages of injured plants should have been obtained with more than 1 insect per plant.

Other tests were made with larger plants but it was found that the amount of vein clearing decreased markedly with size of plant after the 10 to 15 leaf stage. Large numbers of leafhoppers were placed on plants with roots 4 to 6 cm. in diameter but no injury of the type observed on smaller plants was noted. Also, no vein clearing was noted on plants on which colonies of the experimental strain were reared. It has been observed that slow growing plants are much less likely to show vein clearing than more rapidly growing ones. If the rate of development of young leaves in older plants is a factor in their failure to respond to introduction of toxin by the insects, is not known.

Further tests were made to determine whether age, stage of development, or sex of the leafhopper was associated with ability to induce vein clearing. In these tests, nymphs of 3 sizes (small, medium, and large), adult males, and adult females were caged singly on rapidly growing sugar beet plants. The number of plants showing injury, and the severity of injury, were

about the same in all lots of plants. Apparently neither age, size, nor sex of insect within the ranges tested appreciably influences severity of injury.

SUMMARY

When nonviruliferous beet leafhoppers from stock cultures or field collections feed on seedling sugar beet plants they occasionally induce a type of vein clearing similar to that produced by curly-top virus. Selection and mating of individuals that induced vein clearing resulted in the production of a strain of leafhoppers in which more than 50 per cent of the individuals are capable of inducing vein clearing.

Injury is restricted usually to leaves on the side of the plant on which the leafhoppers are feeding and appears to result from retardation or inhibition of chlorophyl production in the cells close to the vascular bundles. It has been induced only on young, rapidly growing plants.

One leafhopper appears to be able to induce vein clearing about as severe as that produced by 2, 5, 10, or 20 leafhoppers. Adult males, adult females, and nymphs appear to be about equally effective in production of vein clearing.

U. S. SUGAR PLANT FIELD LABORATORY,
RIVERSIDE, CALIFORNIA

A MECHANISM FOR LIBERATION OF SPORES FROM BENEATH THE BARK AND FOR DIPLOIDIZATION IN CHALARA QUERCINA [1]

J. G. Leach, R. P. True and C. K. Dorsey

Although much has been learned about oak wilt since its recognition as a destructive disease, the method of long distance spread is still not known. Mycelial mats producing conidia were found between the bark and wood in Illinois in 1951 and reported by Curl et al.[2] The mechanism of liberation of the spores was not mentioned although it was stated that the fungus frequently grew along insect galleries.

During April, 1952, a number of oak trees killed by wilt in 1951 in various parts of West Virginia were examined. Mycelial mats, similar to those reported from Illinois, were found under the bark of many trees, producing conidia in profusion. Near the center of each mat were one or more thick cushions with daedaloid contact surfaces (Fig. 1) composed largely of *Chalara* mycelium and a mass of enlarged ovoid fungus cells. It was observed that the mats and cushions were usually associated with cracks in the bark but the significance of the cushions was not recognized until one large tree was cut and the trunk and branches brought in for further observation. When the tree was cut only a few cracks and mycelial mats were observed, but after a few days' exposure in rainy weather new cracks began to develop and it was obvious that the cracks in the bark were made by pressure exerted on the bark by the developing fungus cushions. Figures 2 and 3 show how the expanding fungus cushion cracks the bark and by separating it from the wood forms a large moist chamber in which the gray sporulating mycelial mats develop.

This is a mechanism for liberating the spores that form on the mycelial mats under the bark. Shortly after the cracks are formed in the bark the underlying cavities are invaded by several species of insects.[3] Some of these apparently feed upon the fungus and since they come in intimate contact with spores are all potential vectors of the disease. However, it remains to be shown whether or not they visit healthy trees under conditions permitting infection.

Discovery of the perithecial stage of *C. quercina* and the demonstration of the heterothallic nature of the fungus[4] raised many questions about the methods of diploidization, the factors influencing the formation of perithecia, and the role of ascospores in long distance spread. Barnett, Staley and True[5] have shown that the mycelial mats occurring beneath the cracked bark are ready for spermatization and will produce perithecia within a few days when conidia of the opposite compatibility group are introduced. It is obvious that cracks made in the bark by the expanding cushions not only liberate spores capable of causing infection but also permit insects to transfer spores of opposite compatibility groups to receptive mycelial mats and thus bring about ascospore formation. A distinct and characteristic odor is associated with the mycelial mats and cushions. This odor is apparently attractive to insects and should be considered as an essential part of the mechanism.

[1] Accepted for publication June 13, 1952.
Published with the approval of the director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 458.

[2] Curl, E. A., G. J. Stessel, and B. M. Zuckerman. 1952. Macroscopic growth of the oak wilt fungus in nature. (Abs.) Phytopathology 42: 6.

[3] Principally Coleoptera, Nitidulidae, including *Glischrochilus sanguinolentus* (Ol.), *G. fasciatus* (Ol.), *G. confluentus* (Say), *Carpophilus lugubris* Murr. *Colopterus semitectus* (Say), *C. morio* (Er.) and *Epuraea* spp.

[4] Bretz, T. W. 1952. The perithecial stage of *Chalara quercina* Henry. (Abs.) Phytopathology 42: 3.

[5] Barnett, H. L., John M. Staley and R. P. True. 1952. Mycelial mats of *Chalara quercina* on killed oak trees as a potential source of perithecia in nature. Phytopathology 42: 531-532.

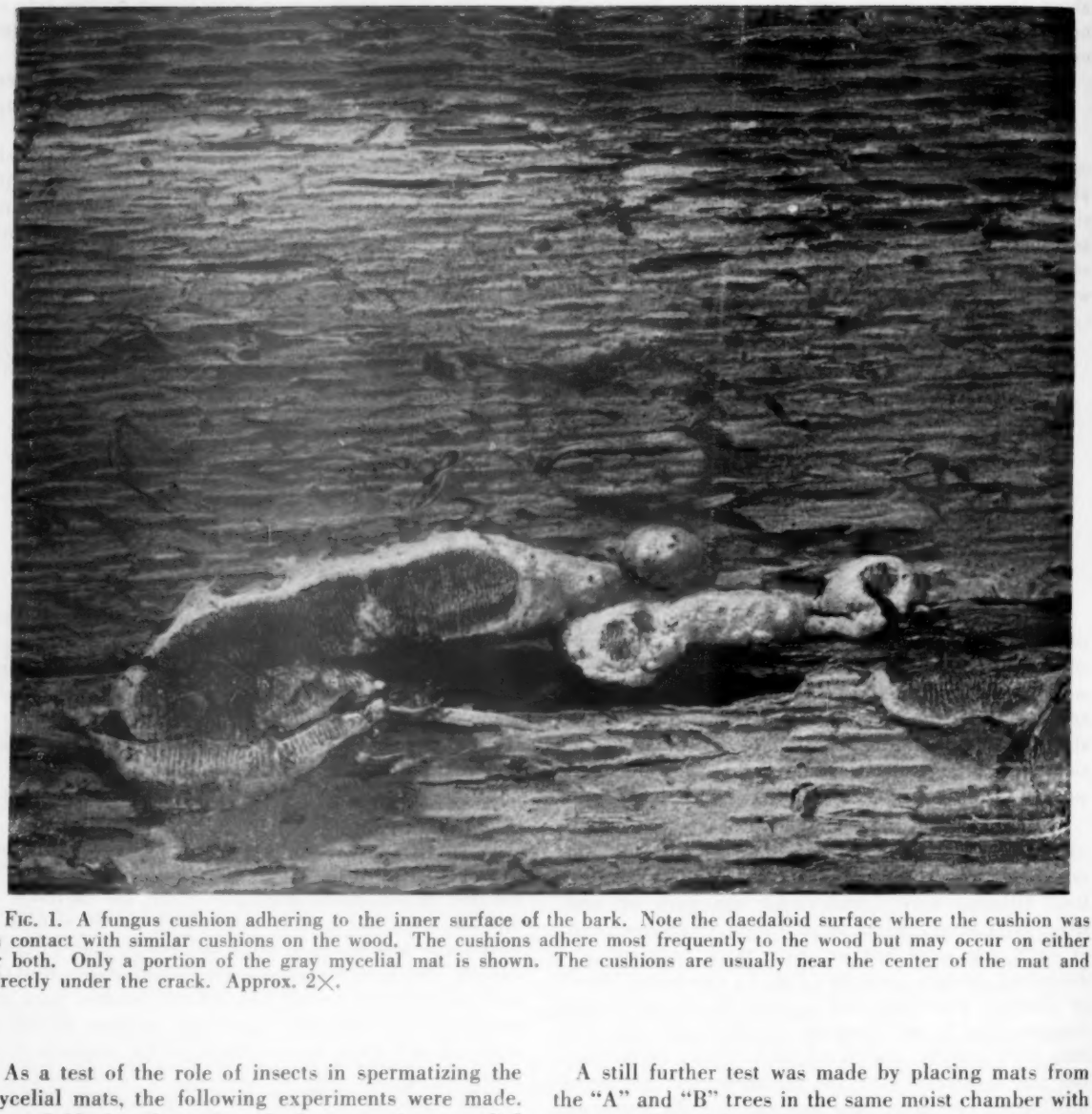


FIG. 1. A fungus cushion adhering to the inner surface of the bark. Note the daedaloid surface where the cushion was in contact with similar cushions on the wood. The cushions adhere most frequently to the wood but may occur on either or both. Only a portion of the gray mycelial mat is shown. The cushions are usually near the center of the mat and directly under the crack. Approx. 2×.

As a test of the role of insects in spermatizing the mycelial mats, the following experiments were made. Several blocks of wood or bark supporting mycelial mats from an isolated tree, killed by wilt in 1951, from which only the A-compatibility strain of the fungus had been cultured, were collected and placed in a moist chamber. Several insects of the species[6] found associated with the mats were allowed to crawl over a petri-dish culture of the B-compatibility strain and then placed in the moist chambers. Three days later perithecia appeared on the mats. No perithecia were formed on mats held in similar moist but untreated chambers.

As a further test insects collected from a tree from which only the B-compatibility strain had been isolated, were placed in a moist chamber with mats from the "A" tree. Again perithecia appeared in 3 days.

[6] *Glischrochilus sanguinolentus* (Ol.), *Epuraea* sp. and an unidentified species of Orthoperidae.

A still further test was made by placing mats from the "A" and "B" trees in the same moist chamber with insects collected from the "A" tree. Here, again, after 3 days perithecia appeared on the "A" mats. No perithecia were observed on the "B" mats but the "B" mats were somewhat older than the "A" mats and were perhaps beyond the best stage for effective spermatization.

During the period of these experiments numerous mats from the "A" tree were observed, both in moist chambers in the laboratory and on the trunk and branches of the tree, and no perithecia were produced except where they were purposely spermatized either directly or through the agency of insects from a tree containing the "B" strain of the fungus or insects artificially contaminated with the "B" strain. The "A" tree used in these experiments was an isolated one at least 5 miles from any other known case of wilt. Al-

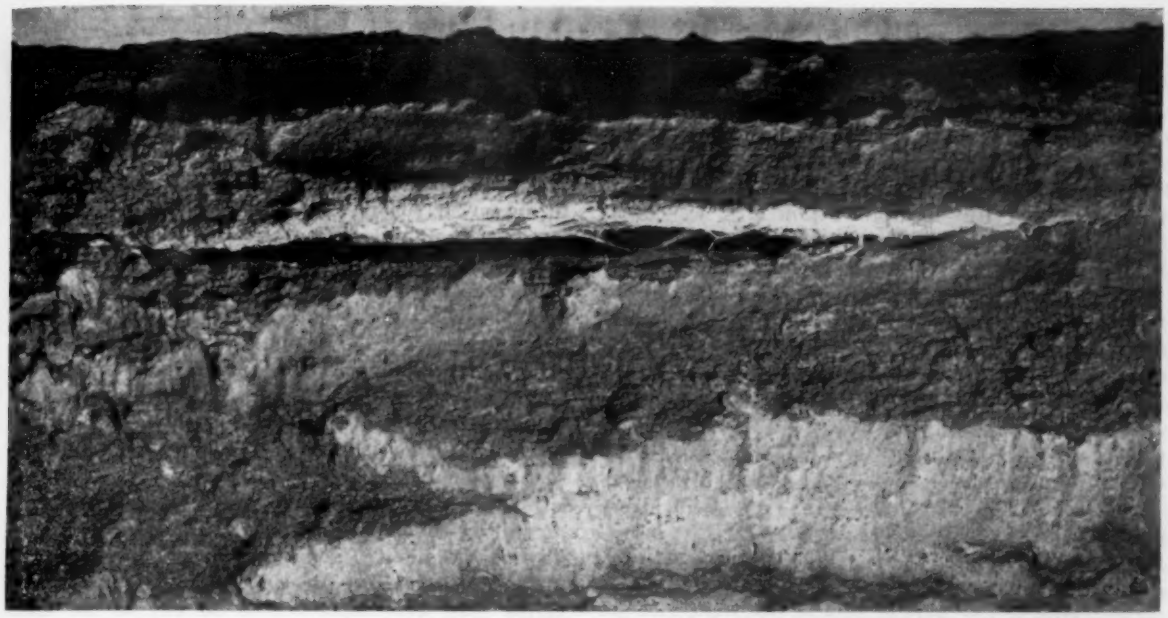


FIG. 2. A fresh crack in the bark of an infected tree showing how the bark has been pushed away from the wood by the fungus cushion providing a moist chamber for the development of the fungus mat. The crack offers access to insects which are apparently attracted by the odor of the fungus. Approx. natural size.

though the mats on this tree were well populated with insects, no perithecia were developed on it in nature. Had there been other infected trees nearby, giving the insects access to conidia of the "B" strain of the fungus, perithecia might have been formed on this tree in nature .

WEST VIRGINIA UNIVERSITY
MORGANTOWN, W. VA.

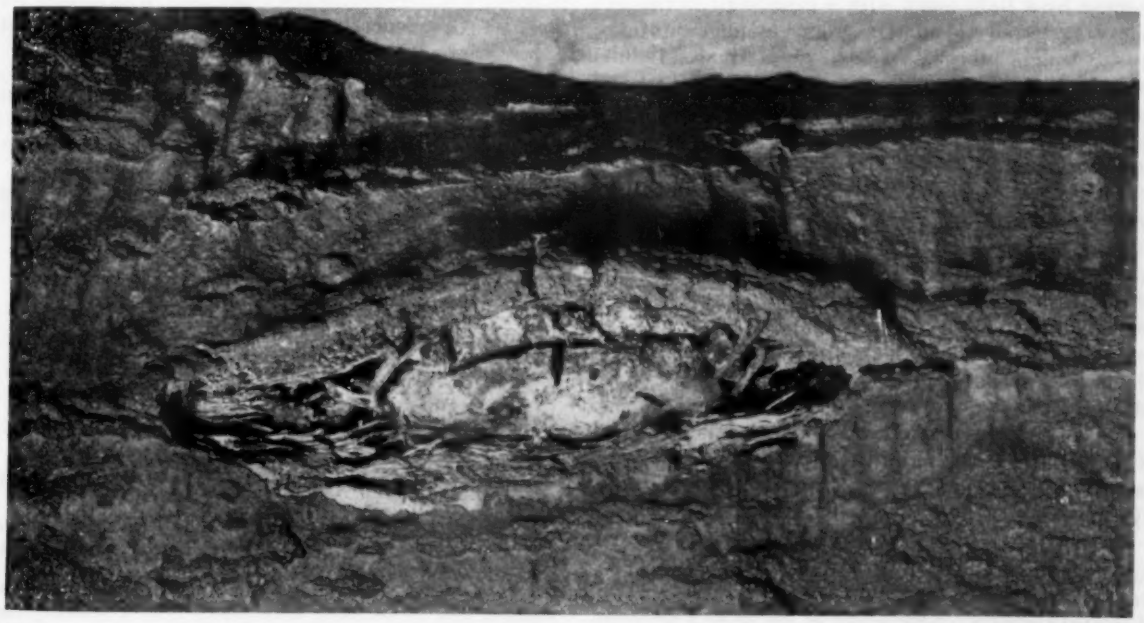


FIG. 3. A crack from which a small segment of bark has been removed to show the large fungus mat which has lifted the bark in a sharp curve. This crack was near a crotch where the bark adhered more tightly and considerable growth pressure was required to lift the bark in this manner. Approx. natural size.

APHID-TRANSMISSION OF POTATO LEAFROLL VIRUS TO SOLANACEOUS SPECIES [1]

Hugh C. Kirkpatrick [2] and A. Frank Ross

In most of the reported studies on transmission of potato leafroll virus (*Corium solani* H.), an aphid-borne persistent virus, the potato (*Solanum tuberosum* L.) has been used as both source plant and test plant (5, 6, 14). These studies have provided valuable information but the use of the potato imposes limitations on the kind and extent of experimentation that can be done. Discovery of suitable test plants for leafroll virus (4, 9, 10) greatly facilitated work with this virus. This report presents results of experiments in which these test plants were used in a study of suscept-virus-vector relationships.

MATERIALS AND METHODS.—All experiments were carried out in a greenhouse kept essentially insect-free by frequent use of insecticides. Seeds were germinated in flats or large pots and the seedlings were transplanted to 3-in. pots as soon as the first leaves developed. The plants were used in transmission studies as soon as they were well established, usually when they were in the 4-leaf stage.

Unless otherwise stated, the insect vector was the peach aphid, *Myzus persicae* (Sulz.). The aphids were colonized in an insectary chamber on cabbage (*Brassica oleracea* var. *capitata* L.), which is immune from leafroll virus (8). In some experiments where viruliferous aphids were needed, the insects were reared in another chamber on infected potato or *Datura stramonium* L. plants. Aphids to be transferred were made to move by a light touch with a camel-hair brush and then transferred to another plant by means of the brush. Unless otherwise stated, only wingless adults were used. When the source plant was infected potato, only these aphids on lower leaves were used. The aphids were not starved prior to acquisition feeding since Watson (19) found that such starving had little or no effect on the ability of aphids to transmit persistent viruses. The test seedlings to which aphids were transferred were covered with 2-x-6-inch Pyrolin cylinders covered on top with fine-mesh cheesecloth. Insects were removed from such plants by means of a camel-hair brush in accurately timed experiments, and by fumigation with nicotine sulfate in others. The test plants then were placed on a greenhouse bench and examined at intervals for symptom development.

Since the number of observations was small in many of the experiments, chi-square (X^2) was calculated only after application of Yates' correction for continuity (16).

SOURCES OF INOCULUM.—*Comparison of species.*—In an earlier report (10) it was shown that *Physalis floridana* Rydb., *Datura stramonium*, and *P. angulata* L. differ in the ease with which they can be infected. Susceptibility varied in the order given, with *P. floridana* the most susceptible. No direct comparisons of these species with the potato have been made, but the results of independent experiments indicate that the potato is less susceptible than the above test plants. It was of interest to determine whether these 4 species vary in the same manner with respect to suitability as source plants.

Aphids (*Myzus persicae*) were reared on infected plants of the 4 species and transferred to *Physalis floridana* seedlings, 1 aphid per plant. Those reared on *P. floridana* consistently were able to infect more plants than were those reared on any of the other species (Table 1). The data suggest that the 4 species vary with respect to suitability as source plants in the same order that they vary in susceptibility, although more data would be needed for a definite conclusion. Whether or not similar results would be obtained with test plants other than *P. floridana* has not been determined by direct test. However, it was common experience to obtain larger numbers of infections in experiments where *P. floridana* was used as test plant than in similar ones in which other test species were used, regardless of the source plant used.

The Physalis species used are poor host plants for aphids. Consequently, in experiments where it was

TABLE 1.—*Infection of Physalis floridana with leafroll virus by aphids reared on various infected species*

Source species[a]	No. test[b] Plants	Plants Infected No.	Plants Infected Per cent
Potato (*Ashworth*)	50	34	68.0
Physalis angulata	50	39[c]	78.0
Datura stramonium	50	41[d]	82.0
P. floridana	50	49[c,d]	98.0
Potato (S41956)	75	25	33.3
D. stramonium	75	30	40.0
D. stramonium	80	27[e]	33.8
P. floridana	80	60[e]	75.0

[a] Aphids reared on source plants.

[b] Single aphids were placed on each test plant and allowed to feed 6 days.

[c] $X^2 = 7.670$. Probability that the data from *P. angulata* and *P. floridana* were drawn from a homogeneous population is less than 0.01.

[d] $X^2 = 5.441$. Probability that the data from *D. stramonium* and *P. floridana* were drawn from a homogeneous population is less than 0.02.

[e] $X^2 = 25.797$. Probability that the data from *D. stramonium* and *P. floridana* were drawn from a homogeneous population is less than 0.01.

[1] Accepted for publication June 16, 1952. Based on a thesis submitted by the senior author to the Faculty of the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Present address: Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Wenatchee, Washington.

TABLE 2.—*Transmission of leafroll virus from excised leaves differing in age*

Source Species	Leaves Location on plant	Test Plants Species	Plants Infected No.[a]	No.	Per cent
Potato[b] (S41956)	Upper	*D. stramonium*	10	2[c]	20.0
	Middle	*D. stramonium*	10	3[d]	30.0
	Lower	*D. stramonium*	10	9[c,d]	90.0
Datura[e] *stramonium*	Upper	*P. floridana*	39	26	66.7
	Middle	*P. floridana*	50	36	72.0
	Lower	*P. floridana*	47	34	73.9

[a] One aphid per plant.
[b] Aphids on source leaves 7 days.
[c] $X^2 = 7.50$. Probability that the data from upper and lower leaves were drawn from a homogeneous population is less than 0.01.
[d] $X^2 = 5.2083$. Probability that the data from middle and lower leaves were drawn from a homogeneous population is less than 0.05.
[e] Aphids on source leaves 48 hours.

necessary to rear aphids on infected plants, either Datura or potato plants were used.

Different leaves from the same plant.—Potato plants, U.S.D.A. seedling 41956, were grown from infected tubers. When the plants were about ready to blossom, leaves from the upper, middle, and lower portions of the stem were removed and placed with their petioles in test tubes containing water. Each leaf was infected with nonviruliferous peach aphids and caged. Seven days later aphids were transferred to Datura seedlings, 1 aphid per plant. The aphids that fed upon the older lower leaves of the source plant caused more infections than did those that fed on the middle leaves (Table 2). The young upper leaves were the poorest sources of inoculum. This is of practical interest for in natural infestations of the potato, *M. persicae* feeds chiefly on the lower leaves.

Datura stramonium leaves were tested similarly except that the aphids were allowed to feed for only 48 hours on the source leaves and *Physalis floridana* seedlings were used as test plants. The 3 types of leaves were approximately equal as sources of inoculum (Table 2).

TABLE 3.—*Transmission of leafroll virus to two Physalis species by three aphid species*

Aphid species[a]	*P. angulata* No. Plants[b] Infected		*P. floridana* No. Plants Infected	
		Per cent		Per cent
Myzus persicae	13/50	26	45/50	90
M. convolvuli	2/10	20	5/50	10
Macrosiphum solanifolii	4/100	4	9/100	9

[a] Reared on infected *Datura stramonium*. Test feeding period was 6 days, 1 aphid per plant.
[b] Numerator is the number of test plants infected. Denominator is the number of test plants used.

TRANSMISSION BY DIFFERENT APHID SPECIES.—Several aphid species have been reported as vectors of leafroll virus. In comparative tests, *Myzus persicae* usually gave the best transmission, although other species transmitted the virus readily under some conditions (5, 11, 12). Three aphid species were collected from potato fields in New York and tested for ability to transmit leafroll virus from Datura to the 2 Physalis species.

Myzus persicae was the most effective vector regardless of the test plant used (Table 3). *M. convolvuli* (Kalt.) was but slightly less effective when the test plant was *Physalis angulata* but when *P. floridana* was used, *M. persicae* was much the better vector. *Macrosiphum solanifolii* (Ashm.) was approximately equal to *Myzus convolvuli* in ability to transmit to *P. floridana* but was considerably poorer in ability to transmit to *P. angulata.*

In tests with *Myzus persicae* reared on infected plants, adults were better vectors than young nymphs but slightly poorer than intermediate nymphs. However, relative transmission percentages by the 3 groups varied with the source plant and with the test plant. It is probable that the differences observed were due at least in part to differences in length of acquisition feeding by the different age groups and to the tendency of adults to move about.

EFFECT OF NUMBER OF INSECTS ON TRANSMISSION.—There are 2 possible explanations for the fact that transmission is obtained more readily with large numbers of insects than with 1 or a few. One explanation is that insects can inject subminimal doses into plants and that several of these can combine within the plant and cause infection. Thus it would be possible for a colony to produce infection regardless of whether or not any of the members could have produced it alone. The alternate explanation is that infections are local and independent and that the probability of infection by a colony is the probability that the colony contains at least 1 insect capable of causing infection if alone. Watson (18) and Storey (17) obtained strong evidence in support of the latter. Watson (18) pointed out that if infections are local and independent the following equations should be valid:

$$P = 1 - q^x \quad \text{.....(1)}$$
$$p = 1 - q \quad \text{.....(2)}$$

where p is the probability of infection by one insect, P is the probability of infection by x insects and q is the probability of noninfection by a single insect. Use was made of these equations in the interpretation of the data reported in this section.

Effect of varying the number of viruliferous insects.—Insect levels of 1, 5 and 10 aphids were used. The insects were reared on infected plants and tested on seedlings of *Physalis angulata.* Long acquisition feeding periods were used to minimize differences in amount of virus picked up by the aphids. Six-day test feeding periods were used to insure detection of all aphids capable of transmitting the virus. When a test feeding period of approximately 6 days was used, infec-

TABLE 4.—*Transmission of leafroll virus to Physalis angulata by 1, 5, and 10 aphids*

Expt.	No. of[a] aphids		Test Feeding	No. of[b] Plants	P[c]		p[d]	
	V	NV			Obs.	Calc.	Obs.	Calc.
			Hrs.					
	1	0	144	9/20			.450	
1	5	0	"	14/20	.700	.950		.214
	10	0	"	18/20	.900	.998		.205
	1	0	144	9/25			.360	
	5	0	"	21/25	.840	.893		.302
2	10	0	"	22/25	.880	.989		.191
	1	4	"	8/25			.320	
	1	9	"	4/25			.160	
	1	0	144	17/40			.425	
	5	0	"	27/40	.675	.937		.211
3	10	0	"	35/40	.875	.996		.188
	5	5	"	18/40	.450	.937		.113
	1	9	"	8/40			.200	
	0	10	"	0/40				.000
	1	0	120	13/30			.433	
	5	0	"	20/50	.400	.942		.097
4	10	0	"	46/50	.920	.997		.223
	1	0	24	3/30			.100	
	5	0	"	11/50	.220	.410		.048
	10	0	"	41/50	.820	.651		.157

[a] Number of aphids placed on each test plant. V denotes that the aphids were reared on infected potato or Datura and NV that they were nonviruliferous (reared on cabbage).

[b] Numerator is number of test plants that became infected and the denominator is the number of test plants used.

[c] Observed and calculated probability of infection by each colony. Latter obtained from equations 1 and 2.

[d] Observed and calculated probability of infection by single aphids. The former is that observed for aphids feeding alone. The latter was calculated from equations 1 and 2 and represents the probability of any one member of the colony infecting a plant.

tions resulting from the feeding of 5 and 10 aphids were invariably less than expected, calculated by equation 1 (Table 4). In the 4 experiments, a total of 8 such calculations was possible. If equation 1 were strictly valid, the probability of every observed value (P-Obs.) being lower than the corresponding calculated one (P-Calc.) would be $(\frac{1}{2})^8$ or 0.0039. It is evident that in these tests, the probability of infection by a colony was less than the probability that the colony contained at least 1 transmitter, i.e., some colonies failed to infect the test plants despite the fact that they almost certainly included at least 1 individual that could have infected a plant had it been feeding alone. The reduced efficiency of individuals when feeding with others is expressed quantitatively by the difference between the observed and calculated values of p. In each of the 4 experiments, a viruliferous aphid feeding as a member of a colony of 10 was approximately half as effective as one feeding alone. In 3 of the 4 experiments an aphid feeding as a member of a colony of 5 was more effective than one feeding as a member of a colony of 10.

If infections are not local and independent, the observed values of P should be greater than the calculated values, for aphids unable to infect when feeding alone would contribute to the ability of colonies to infect. The data do not justify abandonment of the concept of local and independent infections. Regardless of the explanation accepted, the observed values are lower than would be expected. It is evident that in the experiments reported there were other factors in operation that tended to prevent systemic infection of plants colonized by 5 or 10 aphids. One such factor might be that the test plants vary in ease of infection, a few being very difficult or impossible to infect; thus the calculated values approaching 100 per cent could not possibly be obtained. Also, an aphid forced to feed on a plant along with others may not feed as long in one spot or as regularly as if it were feeding alone. A third possible factor is that the feeding of several insects on a plant may induce changes in the plant that cause it to be more resistant to infection or which prevent systemic spread of the virus. Since small seedlings were used, colonies of 5 and 10 aphids represent rather high infestation levels.

Tests such as experiment 4 (Table 4) should provide evidence of interference in feeding, for with nonstarved aphids interference should be as important in short test feedings as in long ones. While the results of a single test are insufficient to justify definite conclusions, they suggest that interference in feeding is not of great importance and that other factors are involved. Interference should be most pronounced in the 10-aphid colonies, yet such colonies that fed only 24 hours on the test plants actually infected more plants than expected while similar colonies that fed 120 hours infected fewer than expected.

Transmission by mixed colonies of viruliferous and nonviruliferous aphids.—In experiment 2 (Table 4) single viruliferous aphids forced to feed with 4 nonviruliferous aphids produced only slightly fewer infections than did those feeding alone, but those forced to feed with 9 nonviruliferous aphids produced less than half as many infections as did those feeding alone.

In experiment 3 similar results were obtained with single viruliferous aphids. Again, those allowed to feed alone caused over twice as many infections as did those forced to feed with 9 nonviruliferous aphids. Colonies of 5 viruliferous aphids were similarly more effective than colonies of 10 containing but 5 viruliferous aphids. By the chi-square test, the probability of the above differences occurring in a homogeneous population is less than 0.05 if the data are not corrected and slightly greater than 0.05 if Yates' correction for continuity is applied (16). The differences become of greater significance when the relative abilities of individual insects to infect (p) are compared. Quantitatively, the reduction in efficiency of individual vectors was approximately the same as that calculated for individual members of colonies of the same size where all members had equal acquisition opportunities. In general, this reduction in efficiency was greater in a 10-aphid colony than in one of 5. It is apparent that an aphid capable of infecting a plant when feeding

alone is less likely to do so if the plant is infested with other aphids. These results cannot be explained on the basis of variation in susceptibility among the test plants. In experiments 2 and 3, the number of plants expected to be infected by the mixed colonies was in each case equal to the number actually infected by single aphids. Hence, the expected numbers of infections were not beyond an unattainable limit.

Although insufficient data were obtained to prove that the reduction in individual efficiency is not due to feeding interference, the results discussed in the preceding section indicate that interference is not the only factor involved and that the reduction in efficiency is in some manner dependent upon the use of relatively long feeding periods on the test plant. It appears probable, therefore, that the effect is partly upon the plant. Aphid-feeding over a 6-day period may so alter the physiology of the plant as to increase resistance to infection, or so injure the phloem tissues as to prevent movement of the virus from some areas and result in localization of the virus. If either or both occur, the tendency of plants to escape systemic infection would increase with time. Aphids that normally would be able to cause infections late in the test period would be unable to do so.

EFFECT OF LENGTH OF FEEDING ON TRANSMISSION.—Elze (6) reported that aphids (*Myzus persicae*) that had fed upon infected potatoes for 14 hours were able to infect healthy potatoes during a 24-hour test feeding but not during a 10-hour feeding. Smith (14) found that aphids fed on infected potatoes for 6 hours were able to infect healthy potatoes during a 48 hour test feeding but not in one of 24 hours. Aphids fed on infected plants for 2 hours did not become infective. Aphids that had fed 7 days on infected plants were able to infect healthy plants in periods as short as 2 hours. The data of Elze suggest a latent period in the vector of between 24 and 38 hours while those of Smith suggest one of between 30 and 54 hours. Data on the length of time required for *Myzus persicae* to pick up the virus and transmit it to *Physalis floridana* were obtained in the experiments described in the following sections.

Acquisition feeding.—Aphids were placed on Datura plants that had been infected for at least 30 days. At the intervals indicated in Table 5, 10 aphids were transferred to *Physalis flordana* seedlings, 1 aphid per plant. Consecutive transfers of each aphid to another healthy plant were made at 24-hour intervals until most of the aphids were dead.

Three of the 10 aphids allowed to feed on the infected plant 30 minutes became infective (Table 5). As the feeding period was increased, up to about 24 hours, the number of aphids that became infective also increased. Aphids that became infective after feeding for periods of 8 hours or less were poor transmitters as evidenced by the fact that they were able to infect but few plants. Those allowed to feed for 12 hours or longer were good transmitters as they infected more than half the test plants upon which they fed.

No evidence of multiplication of the virus within the vector was obtained. If such occurs, it is at a rate too slow to be detected during 11 consecutive transfers of 24 hours each and is of much less importance in determining the ability of an aphid to infect plants than is the amount of virus picked up during acquisition feeding.

Length of time on healthy plants.—Aphids reared on infected Datura were transferred, a single aphid per plant, to *Physalis floridana* seedlings and allowed

TABLE 5.—*Transmission of leafroll virus to Physalis floridana by Myzus persicae allowed to feed for different periods on infected Datura stramonium*

Expt.	Acquisition Feeding	Plants infected by all [a] tested insects		No.[b] infective insects	Plants infected by infective insects		Max. no. plants[c] infected by single insect
	Hrs.	No.[c]	Per cent		No.[c]	Per cent	
1	Continuous	44/93	47.0	10/10	44/93	47.0	7/10
	24	25/67	37.4	6/10	25/52	48.0	9/10
2	Continuous	34/67	51.0	10/10	34/67	51.0	6/7
	48	24/40	60.0	9/10	24/38	63.2	4/7
	24	40/56	71.5	9/10	40/51	78.5	6/7
	12	23/52	44.3	6/10	23/35	65.8	5/7
3	12	16/57	28.0	5/10	16/34	47.0	8/11
	10	10/50	20.0	6/10	10/41	24.4	4/11
	8	4/45	8.9	3/10	4/17	23.5	2/11
	6	3/49	6.1	2/10	3/22	13.6	2/11
4	4	8/72	11.1	6/10	8/53	15.0	2/11
	2	5/72	5.9	5/10	5/42	12.0	1/11
	1	4/94	4.3	4/10	4/36	11.0	1/11
	½	5/84	6.0	3/10 [d]	5/22	23.0	2/11

[a] Aphids tested singly, 24-hour test feeding period.
[b] Numerator is number of aphids found infective and the denominator the number of aphids tested.
[c] Numerator is number of plants infected and the denominator the number of plants fed upon.
[d] Two of the aphids infected the plants fed upon during the first 24-hour period.

TABLE 6.—*Transmission of leafroll virus by aphids reared on infected Datura stramonium and allowed to feed for different length periods on Physalis floridana*

Expt.	Test feeding period	Plants infected [a] No.	Per cent
1	8 days	45/50	90.0
	4 days	42/50	84.0
	2 days	39/50	78.0
	1 day	40/50	80.0
2	12 hours	11/50	22.0
	8 hours	17/47	37.0
	4 hours	9/47	19.0
3	2 hours	19/50 [b]	38.0
	4 × ½ hours [c]	12/40	30.0
	½ hour	7/50 [b]	14.0

[a] One insect per plant.
[b] $X^2 = 6.289$. Probability of this difference occurring in homogeneous population is less than 0.02.
[c] Four consecutive interrupted half-hour periods on the same plant.

to feed for specified periods, the shortest of which was 30 minutes. Of the 50 aphids allowed to feed for this short period, 7 caused infection (Table 6). The number of infections increased as the feeding time was lengthened up to 24 hours. Four consecutive interrupted half-hour periods resulted in more infections than single half-hour periods and only slightly fewer infections than a single uninterrupted 2-hour period.

It is to be expected that the longer the test feeding period, the greater will be the probability of transmission. The main point established is that, under the conditions used, infection can result from test feedings as short as 30 minutes.

In tests with *Physalis angulata* as test plant, the number of successful transmissions was low even with periods up to 32 hours. The shortest period during which transmission occurred was 4 hours.

Latent period in the vector.—In the experiments reported in Table 5, some aphids picked up the virus from one plant and infected another within a 24.5-hour period. Tests were made to see if infection could be established in still shorter periods under conditions optimal for transmission.

Nonviruliferous aphids reared on cabbage were transferred to infected *P. floridana* and allowed to feed 30 minutes. Immediately following the acquisition period, groups of 5 were transferred to each of 5 *Physalis floridana* seedlings. Each colony was serially transferred to another *P. floridana* seedling at half-hour intervals for 10 transfers. All test plants were fumigated at the end of the test feedings.

One colony infected the second test plant fed upon, another the sixth, another the seventh and another the eighth (Table 7). Thus 1 colony picked up the virus from 1 plant and infected another within an elapsed time of 1.5 hours while 4 of the 5 colonies were able to do this within 4.5 hours. It is very improbable that the aphids acquired the virus prior to the start of this experiment. In numerous other tests, aphids similarly reared on cabbage, which is immune from the virus, and tested on Physalis or Datura were uniformly nonviruliferous. Hence, when a good source plant and a highly susceptible test plant are used, the mimimum latent period of leafroll virus in *Myzus persicae* is less than 1.5 hours, a period much shorter than that previously reported from experiments on potatoes.

DISCUSSION.—Carsner and Lackey (2) and Giddings (7) concluded that resistant sugar beet varieties require a larger virus dose for infection, and when infected carry a lower concentration of curly top virus than do similar plants of susceptible varieties. The same may be true for leafroll virus and could explain the observed correlation between suitability of a species as source of virus and the ease with which plants of the species can be infected. In other words, infected *Physalis floridana* plants may have an unusually high virus concentration and healthy ones may become infected following introduction of very small amounts of virus. On the other hand, the correlation noted above could be explained equally well as due to variation among the susceptible species with respect to the readiness with which the aphids reach virus-bearing tissue in infected plants and susceptible tissue in healthy ones. Presumably, the tissue concerned in both cases is the phloem.

If the 2 Physalis species used in the comparative test of aphid species (Table 3) differed only with respect to the amount of virus required to infect, a given plant species should be the easier to infect with all of the aphid species if it is the easier one to infect with any one aphid species. Since *P. floridana* gave the highest number of infections with 2 species of vector and *P. angulata* gave the highest number with the other vector species, it would appear that factors determining the readiness with which the vector can reach the phloem or other tissue involved are important in determining the ease of infection of the test plants. If this is true, then it would be expected that the feeding habits of the vector also constitute an important factor in determining the relative ease with which virus can be acquired from the several plant species.

TABLE 7. *Transmission of leafroll virus by Myzus persicae fed on infected Physalis floridana plants for 30 minutes and then on healthy P. floridana seedlings for consecutive 30-minute periods*

Colony	No. Aphids	Transmission during consecutive 30-minute test feedings [a] 1	2	3	4	5	6	7	8	9	10
1	5	—	—	—	—	—	—	—	—	—	—
2	5	—	+	—	+	—	—	—	—	+	+
3	5	—	—	—	—	—	+	+	—	—	+
4	5	—	—	—	—	—	—	—	+	—	—
5	5	—	—	—	—	—	—	+	—	—	—

[a] No infection in plants not colonized. Two of five colonies kept on test plants for 5½ hours were able to infect.

There is good evidence that the latent period of viruses in leafhopper vectors represents the time needed for multiplication of the viruses in the insect in some cases and for the circulation of the viruses through the insects' bodies in others. There is no direct evidence that either is true for aphid-borne viruses. Neither would appear to explain results reported here and elsewhere with leafroll virus. Transmission was effected in a total of 1.5 hrs. elapsed time from the beginning of the acquisition period to the end of infection feeding. This period is too short for appreciable multiplication of the virus in the insect. The actual length of the period varies over a wide range, depending at least in part upon the plants used. It is shortest when good source plants and easily infected test plants are used, longest with poor source plants and resistant test plants. These results appear inexplicable on the basis that there is a latent period in the vector during which the virus must pass through the gut wall into the blood and then to the salivary glands. Differences in the amount of virus imbibed by the aphid and the sensitivity of the test plant might be expected to cause minor variations in the time required for the above sequence but not variations so large as those reported.

From data on the transmission of sugar beet yellows virus by *Myzus persicae*, Watson (20) concluded that there is no fixed latent period during which infection could not be obtained provided sufficient trials were made, but rather a period of low but increasing probability of infection. The data in the present report indicate an identical situation in the case of leafroll virus. A possible explanation of this period of low but increasing probability of transmission is suggested by the conclusion that its duration is largely dependent upon the general level of ability of the aphids to transmit under the conditions used. It is suggested that immediately following transfer of an aphid from a diseased plant to a healthy one there is a period of irregular feeding and hence of low probability of transmission. Most experiments designed to determine the length of the latent period employ short acquisition periods and also short test feeding periods. Hence, the probability of infection during any single test feeding is quite low. One factor that will determine whether or not infection is established will be whether or not the aphid feeds long enough in one spot to reach susceptible tissue. When an aphid is transferred from the plant upon which it was reared to a diseased plant and subsequently to a series of healthy plants, the conditions under which it is forced to feed are changed. The incentive to reach the pholem and to feed regularly in the phloem may not be so great as it was during previous periods. The aphid may not like the new food plant as well as the old and will wander about seeking better feeding sites. The mere act of transfer may so disturb the aphid that it will not feed normally. Any or all of these factors could so affect the aphid during the first test feedings that it does not settle down and feed in one spot sufficiently long to inject infective doses into susceptible tissue. During later periods the aphid may become adjusted to the new food plants or become sufficiently starved to settle down and feed in one spot. Since the probability of infection during any one test period is low anyway, the alteration in feeding habits during the adjustment period could easily be sufficient to reduce the probability to zero.

In the experiment reported here (Table 7) conditions were used such that the probability of infection during short test feedings was rather high. A test plant was used in which susceptible tissue apparently is quite readily reached by the vector. Any disturbance in feeding habits during the first several test feedings would have had to be quite marked to prevent the aphids from reaching susceptible tissue during the test feeding and to reduce the probability of infection to zero for any prolonged period of time. There was apparently a short period of adjustment for no colony caused infection during the first period and only one caused infection during the first 5 periods. Infection during the first period might have been obtained had more colonies been tested or had the general level of infectivity been higher. That transmission can be obtained during the first half-hour the aphids are on healthy plants is shown by the data for experiment 3 in Table 6. Seven of the 50 aphids reared on infected Datura were able to infect *Physalis floridana* seedlings during the first half-hour following transfer to the healthy plants. Presumably the probability of infection in this experiment was such that any reduction during the adjustment period was not enough to prevent infection. Reports of the existence of latent periods regardless of the length of acquisition feeding (1, 3, 19) might be due to the use of such conditions that the change in feeding habits during the adjustment period was sufficient to prevent infection.

The suggested hypothesis makes unnecessary the postulation of different mechanisms for the transmission by aphids of persistent viruses and of nonpersistent viruses. Differences in the conditions requisite for transmission of the 2 could be due in part to differences in the concentration of the respective viruses in the plant and to different rates of inactivation of the viruses in the vector, as suggested by Smith and Lea (15). In addition, the differences may be due in part to the ability of an aphid to acquire a nonpersistent virus and then to transmit it to another plant regardless of the tissue in which it feeds. In the case of persistent viruses, neither apparently can be accomplished readily unless the aphid feeds in specific tissues.

Simpson and Shands (13) suggest that direct aphid damage may produce a condition in potato plants that makes them difficult or impossible to infect with leafroll virus. The data on infection by colonies of 1, 5 and 10 aphids (Table 4) suggest that the same may be true for *Physalis angulata*. If this is found to be generally true, it may have a direct bearing on the many reported failures of insecticides to prevent the spread of aphid-borne viruses. It may be that the

use of insecticides to keep aphid infestation levels below that which results in direct aphid injury tends to keep the plants susceptible.

SUMMARY

When *Physalis floridana* and *P. angulata* seedlings were used as test plants, *Myzus persicae* was a more effective vector of leafroll virus than *M. convolvuli* or *Macrosiphum solanifolii*. In further tests with *Myzus persicae*, *P. floridana* was a better source of inoculum than *Datura stramonium*. *P. angulata* was a poorer source than either of the above but better than potato. The older leaves of potato were better sources of the virus than the younger ones.

On *P. angulata*, the probability of infection by a colony of insects during a 6-day feeding period was consistently less than the probability that the colony contained at least 1 insect capable of causing infection if alone. Viruliferous individuals feeding alone infected more plants than did similar ones that fed together with 5 or 10 nonviruliferous aphids. The data are interpreted as indicating that aphid feeding causes changes in the plants that make them resistant to systemic infection.

Some aphids were able to transmit leafroll virus following 30-minute acquisition periods on infected *D. stramonium*. Aphids reared on diseased plants infected *P. floridana* during 30-minute test feeding periods. When any test feeding period was lengthened up to about 24 hours, the number of infections increased. Aphids allowed to feed on infected *P. floridana* for 30 minutes were able to infect healthy *P. floridana* seedlings within an hour after leaving the infected plants. It is suggested that there is no fixed latent period of leafroll virus in its insect vector but rather a period of low probability of infection resulting from altered feeding when the aphids are moved to different food plants.

DEPARTMENT OF PLANT PATHOLOGY
CORNELL UNIVERSITY
ITHACA, NEW YORK

LITERATURE CITED

1. BAWDEN, F. C. 1950. Plant viruses and virus diseases. 3rd ed. 335 pp. Chronica Botanica Co.
2. CARSNER, E., AND C. F. LACKEY. 1929. Mass action in relation to infection with special reference to curly top of sugar beets. (Abs.) Phytopathology **19:** 1137.
3. CHAUDHURI, R. P. 1950. Studies on two aphid-transmitted viruses of leguminous crops. Ann. Appl. Biol. **37:** 342-354.
4. DYKSTRA, T. P. 1933. Weeds as possible carriers of leaf roll and rugose mosaic of potato. Jour. Agr. Res. [U.S.] **47:** 17-32.
5. DYKSTRA, T. P., AND W. C. WHITAKER. 1938. Experiments on the transmission of potato viruses by vectors. Jour. Agr. Res. [U.S.] **57:** 319-334.
6. ELZE, D. L. 1927. De Verspreiding van Virusziekten van de Aardappel (Solanum tuberosum L.) door Insekten. Meded. v. d. Landbouwhoogeschool, Wageningen. Meded. 32. 90 pp.
7. GIDDINGS, N. J. 1946. Mass action as a factor in curly-top-virus infection of sugar beet. Phytopathology **36:** 53-56.
8. HELSON, G. A. H., AND D. O. NORRIS. 1943. Transmission of potato virus diseases. III. Susceptibility of Cruciferae to potato leaf roll virus. Jour. Counc. Sci. Indust. Res. (Australia.) **16:** 261-262.
9. HOVEY, C., AND R. BONDE. 1948. Physalis angulata; a test plant for the potato leafroll virus. Phytopathology **38:** 505-507.
10. KIRKPATRICK, H. C. 1948. Indicator plants for studies with the leafroll virus of potatoes. Amer. Pot. Jour. **25:** 283-290.
11. MCKAY, M. B., AND T. P. DYKSTRA. 1932. Potato virus diseases. Oregon investigations 1924-1929. Ore. Agr. Exp. Sta. Bul. 294.
12. MURPHY, P. A., AND R. M'KAY. 1929. The insect vectors of the leaf-roll disease of the potato. Sci. Proc. Roy. Dublin Soc. **19:** 341-353.
13. SIMPSON, G. W., AND W. A. SHANDS. 1949. Progress on some important insect and disease problems of Irish potato production in Maine. Maine Agr. Exp. Sta. Bul. 470.
14. SMITH, K. M. 1931. Studies on potato virus diseases. IX. Some further experiments on the insect transmission of potato leaf-roll. Ann. Appl. Biol. **18:** 141-157.
15. SMITH, K. M., AND D. E. LEA. 1946. The transmission of plant viruses by aphids. Parasitology **37:** 25-37.
16. SNEDECOR, G. W. 1946. Statistical methods. 4th ed. 485 pp. Iowa State College Press
17. STOREY, H. H. 1938. Investigations of the mechanism of the transmission of plant viruses by insects. II. The part played by puncture in transmission. Proc. Roy. Soc. (London), B, **125:** 455-477.
18. WATSON, M. A. 1936. Factors affecting the amount of infection obtained by aphis transmission of the virus Hy. III. Phil. Trans. Roy. Soc. (London), B, **226:** 458-489.
19. WATSON, M. A. 1946. The transmission of beet mosaic and beet yellows viruses by aphides: a comparative study of a non-persistent and a persistent virus having host plants and vectors in common. Proc. Roy. Soc. (London), B, **133:** 200-219.
20. WATSON, M. A. 1940. Studies on the transmission of sugar-beet yellows virus by the aphis, Myzus persicae (Sulz.). Proc. Roy. Soc. (London), B, **128:** 535-552.

BLACK THREAD OF HEVEA BRASILIENSIS IN LIBERIA [1]

Ellis F. Darley and Savel B. Silverborg [2]

Among the several diseases of *Hevea brasiliensis* (H. B. K.) Muell—Arg., the tree from which most of the natural rubber throughout the world is obtained, are those that affect the tapping panel, the delicate tissues of the inner bark exposed during the tapping process. One of these diseases, known as black thread, black stripe, or black line canker, described by Petch (9), Sharples (10), and others, is common in most of the rubber growing regions of the world. In brief, the cortex exposed by the tapping process is invaded by a parasitic fungus, which progresses down to the cambium and kills it.

In healthy trees, the tapped bark regenerates in a normal way and the panel may be tapped again in 6 or 8 years. Panels with a light infection of black thread heal over so that there is very little trace of the disease in 1 or 2 years and the bark is easily retapped. With severe infections portions of the resulting wounds may never heal, thus rendering that portion of the panel untappable; or if the wound does eventually heal, the regenerated bark is rough, warty, and difficult to retap.

In 1940, panel diseases created new interest on the Firestone Plantation in Liberia, when panels on trees of clone Tandjong Kemala 12[3] were "opened," i.e., tapped for the first time. Panel infection on these trees was much more severe than usual. In addition to the narrow, vertical black lesions, the bark was attacked in patches that in many cases involved the full width of the panel. Approximately 70 per cent of the trees in the area planted with this clone were affected. The disease seemed to be restricted to clone T.K.12.

It appeared that either this was a new disease or a new manifestation of black thread. An investigation was therefore made to determine the cause, its relation to black thread, and to test new fungicides for its control.

LITERATURE REVIEW.—Black thread was first reported in Ceylon in 1909 (9) and in the following years it became prominent in Java and Sumatra. In 1916 the disease was reported in Malaya (12). Rutgers (see 12) in Java in 1914 produced typical black thread symptoms by inoculating panels with *Phytophthora faberi* Maubl. isolated from diseased pods of cacao; in 1916, Dastur (4) produced the same disease on rubber trees with a species of *Phytophthora* obtained from diseased fruits of rubber. McRae (7, 8) described a new species, *Phytophthora meadii*, which caused symptoms similar to black thread when inoculated on the tapping panel. Sharples, et. al., (12) produced the disease in Malaya with an unknown species of *Phytophthora* isolated from tapping panels affected with black thread. In 1929, Thompson isolated what he considered to be 3 species of *Phytophthora* from black thread panels: *P. palmivora* Butler, *P. meadii*, and a new species he named *P. Heveae* (15). Tucker (16) and Leonian (6), in their studies on the genus *Phytophthora*, include *P. faberi* and *P. meadii* under *P. palmivora*. The latter is now generally accepted as the cause of black thread in all rubber growing regions.

Variations from usual black thread symptoms, wherein larger patches of renewing bark were involved, as in the case on clone T.K.12, have been observed in eastern rubber growing countries. Sharples noted that in 1 locality in Malaya the panel was attacked in patches just above the cut and progressed down the panel with the tapping. Pratt (see 10) found the same thing in Sumatra; the individual lines were not apparent and the whole tapping cut turned black.

In 1944, Ford (5) reported symptoms on panels of trees in Ceylon similar to those in Liberia on clones of T.K.12, AVROS 50, and Bodjong Datar 5. The renewing bark just above the cut was attacked in patches and in some cases these coalesced, resulting in large wounds. Inoculations were made in 2 localities with a *Phytophthora* isolated from clone MK 3/2. In 1 locality superficial symptoms developed which were similar to the original disease while in the other locality the symptoms were more nearly like those of the usual black thread.

OCCURRENCE AND NATURE OF BLACK THREAD IN LIBERIA.—In the Harbel area of the Firestone Plantations, black thread occurs, generally, over most of the seedling area. Among clones, infection has been found over an area more than 1000 acres, localized to 40 or 50 acres, or even to as few as 50 or 60 trees.

Black thread is considered a wet season disease. At Harbel, Liberia, the wet season lasts from May to October, with increasing rainfall and fewer hours of bright sunlight (Fig. 1). While the monthly rate of rainfall gradually increases from May to September and then declines rapidly in late October and November, the weekly distribution is variable. Averaging the

[1] Accepted for publication June 21, 1952.

Paper No. 2827, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul, Minnesota.

[2] Formerly Pathologist, Firestone Plantations Company, Harbel, Liberia, now Assistant Pathologist, Division of Plant Pathology, University of California Citrus Experiment Station, Riverside, California; and Research Associate, State University of New York College of Forestry, Syracuse, New York, respectively. The authors wish to acknowledge their indebtedness to the Firestone Plantations Company of Akron, Ohio, for making this investigation possible; to Dr. E. C. Stakman, Dr. K. G. McIndoe, and Dr. C. M. Christensen for their help in the investigation and the preparation of the manuscript; to Dr. C. M. Tucker and Dr. John T. Middleton for identification of some of the fungi studied. A portion of the work was done by the writers at University Farm, St. Paul, Minnesota, and a part by the senior author on the plantations in Liberia in 1946-47.

[3] Also designated as Lampongiana 2.

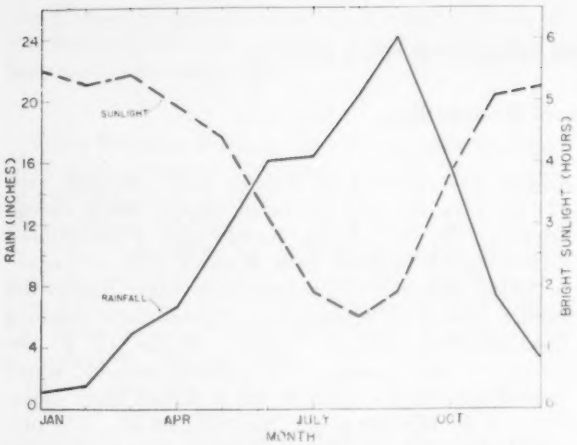


FIG. 1. Average monthly rainfall, 1936-47, and average daily hours of bright sunlight each month, 1936-46 (omitting 1941-42) at Research Department, Firestone Plantations, Harbel, Liberia. (Data compiled by Dr. K. G. McIndoe).

figures for 12 years, 1936 to 1947 inclusive (Fig. 2), there are 2 peaks of rainfall, the 25th week (June 18-24) and the 37th week (September 10-16). Between these 2 peaks there sometimes occurs a period of hot, dry weather known as the "middle dry."

In general black thread first appears in late June or July. The peak of infection is usually reached in late August or September; by the end of October new infections cease and spread of older lesions is usually arrested.

In 1946 and 1947, black thread was first noted about July 10 and June 1 respectively. There was a "middle dry" in 1946 and the peak in severity of disease was not reached until the middle of August. In contrast to 1946, rainfall in 1947 was one of the heaviest on record and the earlier appearance of black thread seemed to be related to the early heavy rain in late May and June. There was no "middle dry" in 1947 and, except for minor fluctuations, there was no decrease in disease until the end of the wet season.

As panel infection develops, lesions can be found that conform in all respects to the typical symptoms ascribed to black thread. The vertical black lines appear on the renewing bark within an inch of the current cut. There may be few or many of these lesions and where numerous they may coalesce to form wider patch-like lesions which result in large wounds. In addition to the usual black thread lesions on T.K.12, larger patch-like lesions, varying from 1 in. in diameter to the full width of the current cut, develop at onset and clearly are not the result of the coalescence of narrower stripe lesions. As tapping is continued, lesions develop into large patches. By the end of the wet season, 2 to 5 in. of panel may be severely injured on 70 to 80 per cent of the trees of the area planted with clone T.K.12 (Fig. 3, A). The large lesions, typical of infection on T.K.12, were also observed in localized areas on a small percentage of trees of clones B.D.5, B.D.10, AVROS 50, and Tjirandji 16 and on old seedling trees (Fig. 3, B, C, D).

On panels with light to moderate infection, small splits from which latex exudes develop in the renewing bark. A pad of coagulated latex usually forms underneath the bark causing it to rise and form blisters ½ to 3 in. in diameter; the wood underneath is discolored.

Occasionally infections on T.K.12 continue to develop downward with the tapping process well into the dry season (Fig. 4, A). Also on AVROS 50 and B.D. 5 of the diseases spread upward during the dry season either into previously tapped and regenerated bark or into virgin bark above the point where the panel was originally opened (Fig. 4, B, C).

ISOLATION OF PHYTOPHTHORA PALMIVORA.—*From Diseased Panels.*—Isolations were made from about 200 tapping panels, 3 of which had been sent to St. Paul, of seedlings and various clones having lesions of the following types: 1) vertical black stripes associated with typical black thread, 2) small to large black patches characteristic of severe infection on T.K.12

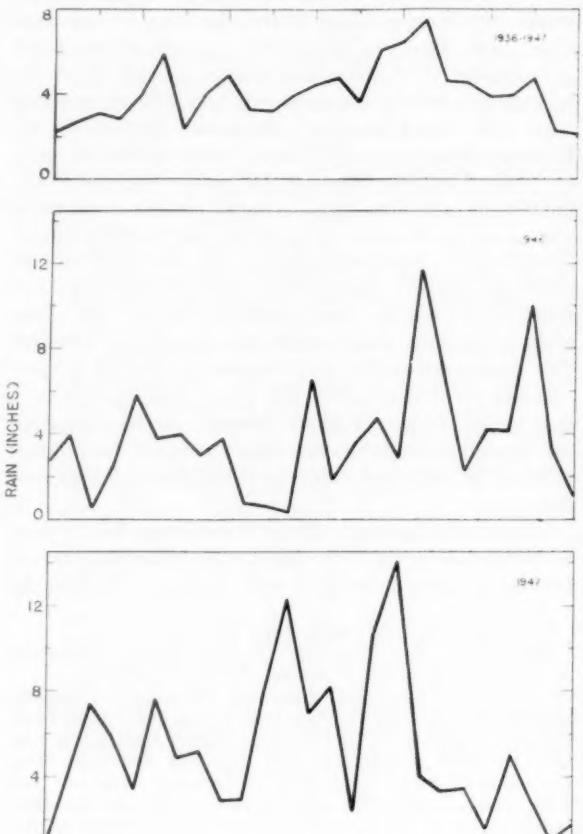


FIG. 2. Average weekly rainfall from the 20th to the 44th week (May 14-Nov. 4), 1936-47 and weekly rainfall for same period for 1946 and 1947 at Research Department, Firestone Plantations, Harbel, Liberia. (Data compiled by Dr. K. G. McIndoe).

FIG. 3. Panels of 4 clones affected with black thread and showing severe patch wounds. A) T.K.12 showing result of two seasons' natural infection. Area of healthy renewed bark between the large upper and lower wounds was tapped during dry months of Nov. 1945 to May 1946. B, C, and D) B.D.5, Tjir. 16, and AVROS 50, respectively. The 1946 wet season infection in B and C was photographed the following dry season. The whiteness of the affected area is due to a palm oil fungicide. Black area on the white is mold growth. Panel D, photographed during the 1946 wet season, shows the large patch wound just above the cut.

and certain other clones, 3) bark which became diseased during the dry season due to spread of infection downward with the tapping or upward into previously renewed, healthy bark, and 4) cracks and blisters appearing in the renewing bark of lightly infected panels.

Small pieces of diseased bark, and wood underneath to a depth of about ¾ in., were surface sterilized successively in 50 per cent alcohol, a 1:1000 solution of mercuric chloride, and sterile distilled water, and placed on potato dextrose or lima bean agar in petri dishes. In 4 or 5 days, fungi grew out from the pieces sufficiently to permit tentative identification. From 50 to 500 such isolations were made from each diseased panel but only a few of these yielded *Phytophthora palmivora*. *Fusarium*, *Trichoderma*, *Penicillium*, and other molds were obtained commonly.

Both the typical and atypical strains of *P. palmi-*

FIG. 4. Panels showing development of disease during the dry season. A) T.K.12. Wound caused by natural infection in 1946 wet season is shown between marks. The disease continued to develop downward on bark tapped into March 1947. Healthy bark on that portion of the panel tapped in April has been scraped away to show lower limits of disease. B) Panel of AVROS 50. Lower diagonal wound between marks is site of 1946 infection. The upper wounds did not appear until April 1947. C) Panel of B.D.5 on which the disease spread upward and killed virgin bark 23 in. above where the panel was originally opened for tapping. Whiteness of affected panel is due to presence of palm oil fungicide.

vora[4] as recognized by Ashby (1) and Tucker (16) were isolated; for convenience these strains are designated as form 1 and form 2, respectively. In addition *Pythium vexans* deBary,[5] cited as the cause of patch canker of rubber, a disease usually restricted to untapped bark near the base of the tree, (11, 15) was isolated from 3 panels.

The vast majority of isolates of *P. palmivora* were form 2, and this form was obtained from all 4 types of lesions. Form 1 was obtained from only 3 panels; from 2 old seedling-tree panels with streak lesions, and from one T.K.12 panel with patch lesions. Form 2 was also isolated from this latter panel. Thus a given form of the fungus was not restricted to a given type of lesion. This suggests that, at least at Harbel, Liberia, a given form of *P. palmivora* may cause the severe infection found on T.K.12 as well as typical black thread streak lesions. *Pythium vexans* may also be involved in panel diseases in Liberia.

From Leaves, Twigs and Fruits.—Second, or abnormal, leaf-fall has been reported from Burma and Ceylon; both leaf-fall and die-back of small branches has been reported from South India (11). In these countries, what is now known as *P. palmivora* was isolated from affected plant parts.

Leaf-fall and die-back of branches occurs on some trees in the wet season in Liberia. In isolations made from recently fallen leaves and their twigs, the discolored base of the petiole was the only symptom. There was no shriveling or wilting of leaves, as observed by McRae (8). About half of the 140 isolates from 150 leaves were form 2 of *P. palmivora* and the rest were *Pythium vexans.*

Of the various fungi obtained from dead twigs only 3 were of form 2 and 16 were form 1. *P. vexans* was not isolated from dead twigs. After 2 months of the dry season more isolations were made from dead twigs and only 1 culture of form 1 of *P. palmivora* was obtained.

Rotted fruits were examined and sporangia were found but isolations yielded no *Phytophthora.*

CULTURAL STUDIES.—Form 1 produces sporangia and chlamydospores profusely and early in culture while form 2 produces few or none. In general form 1 develops a relatively sparse mycelium and form 2 develops an abundant growth. There were, however, variations in the cultural appearance among 17 isolates of form 1 and 39 isolates of form 2 grown on lima bean, potato dextrose, and oat meal agars.

Two isolates of each form were grown at 5° to 35° C. Those of form 1 grew at 10° and those of form 2 started growing at 5° C. The 2 forms developed most abundantly at 20°-30° and 25°-30° respectively. Both grew at 35°, which is a slightly higher maximum than recorded by Tucker (16).

The sporangia of 2 isolates of each form were measured and, as noted by Tucker, those of form 1 were generally larger. The average size for form 1 was $51.5 \times 33.9\ \mu$ and for form 2 was $33.1 \times 26.2\ \mu$.

PATHOGENICITY STUDIES.—*On 1- and 2-year old Seedlings.*—Seedling trees approximately 1-year-old, growing in the greenhouse in St. Paul, were inoculated with 1 culture each of the 2 strains of *P. palmivora* and with *Pythium vexans.* Small wounds, or panels, were made on the trees by scraping a small area of the outer bark to a depth that caused the latex to flow. The inoculum consisted either of disks of mycelium and agar cut from 5-day-old cultures or of a water suspension of sporangia. After inoculation the panels were covered with a moist cotton pad or a moist chamber made of

[4] Identified by Dr. C. M. Tucker.
[5] Identified by Dr. John T. Middleton.

blotting paper and cotton. Sterile agar was applied to panels of control trees.

Within 10 days all the panels inoculated with the 2 forms of *P. palmivora* and *P. vexans* were killed while those on the control trees healed over. The tissues of all inoculated panels turned black. On the stems inoculated with *P. palmivora,* the uninjured bark around the panel turned black and died; in some instances this effect extended as far as 6 in. up and down the stem from the panel. In addition, form 2 generally caused the trees to wilt in from 3 to 6 days while form 1 seldom caused wilting. *P. vexans* caused the wound to turn black but the effect did not extend more than ¼ in. beyond the edge of the wound. None of these trees wilted.

Inoculations were repeated on 1-year-old seedlings at Harbel, Liberia, using the 3 cultures isolated in St. Paul and 23 additional isolates obtained from diseased panels on the plantations. Three of the latter were form 1 and 20 were form 2. The results were essentially the same as those obtained in the greenhouse.

Two-year-old seedlings on the plantations were also inoculated in the same manner with 45 isolates selected from all those obtained from panels, leaves, and twigs. After 10 days the panels of all trees inoculated with both forms of *P. palmivora* were dead. On some of those inoculated with form 2, the uninjured bark surrounding the panel had been killed, while killing on those inoculated with form 1 was restricted to the wounded area. Isolates of form 2 from leaves and twigs were as pathogenic as those from diseased panels. Bark of trees inoculated with *P. vexans* was renewing satisfactorily with no evidence of infection.

On 11-year-old Trees in Tapping.—During the dry season of 1946, trees of 6 clones were inoculated with 1 isolate of form 1 of *P. palmivora* obtained from a diseased seedling panel and 4 isolates of form 2, each secured from a different clone. The inoculum was prepared as follows: The fungus was grown for 5 to 7 days in 250-ml. Erlenmeyer flasks containing 100 ml. of potato-dextrose broth. On the day of inoculation the broth culture was mixed with 100 ml. of 2 per cent water agar and 50 ml. of water in a Waring blendor and poured into a flask fitted with a 2-hole stopper with 2 glass tubes of proper length to allow the inoculum to flow out of 1 of them when the flask was inverted. The contents of 1 flask served to inoculate approximately 40 trees.

Trees were tapped on the half-spiral, alternate 15-day system; that is, on a spiral cut over half the circumference of the tree, tapping each day for 15 days, resting 15 days, and tapping 15 days. Panels were inoculated each tapping day within 1 to 3 hours after tapping for from 1 to 3 tapping periods. The latex was removed from the cut with a stick and the inoculum poured onto the exposed inner bark. A sterile blend of agar and water was poured on the panels of control trees.

In the dry season inoculated panels were covered with moist cotton and cloth bandages to maintain moisture necessary for infection; panels were not covered during the 15-day rest periods. The bandage was not necessary during the wet season.

The development of disease was noted each day, particularly in the first 15-day tapping period. Infection was usually evident in from 3 to 5 days, as a slight discoloration of the renewing bark. By the eighth day definite dark, depressed lesions, of 1 or both of the 2 types described on naturally infected trees, were present. Typical streak lesions were produced on panels inoculated with form 1. Streaks, indistinguishable from those caused by form 1, also developed on panels inoculated with form 2. There also appeared the small to large patch lesions characteristic of natural infection on clones T.K.12, AVROS 50, and B.D.5, developing as such from the beginning and not the result of coalesced streaks. The ratio of patch to streak lesions on a given clone varied from tree to tree but the appearance of at least a few patches was usual on all clones inoculated with the various collections of form 2. On those inoculated with form 1, patches appeared rarely, and then late in the experiment as the result of coalesced streaks.

Fifteen to 25 days after inoculation started, cracks and blisters from which latex exuded, formed on panels inoculated with form 2. No blisters appeared on trees inoculated with form 1.

Final notes on infection were taken at the end of each 15-day tapping period. Recording total percentage of panel area infected both as streak and patch lesions, trees were placed into 4 infection classes: healthy, no infection; light, less than 10 per cent of panel area infected; medium, 10 to 50 per cent infected; and severe, more than 50 per cent infected. Results indicating the 6 clones inoculated and the source of 5 isolates of *P. palmivora,* are in Figure 5. The average percentage of infected panel area appearing as patch lesions only is shown in Figure 6.

Isolates of form 2 were more virulent than those of form 1, because a greater percentage of the trees inoculated with form 2 were in the higher infection classes and a greater percentage of the panel area was killed in patches.

B.D.10 appeared to be the least susceptible of the clones tested. The most severe infection was on T.K.12 with collections of form 2 from B.D.10 and T.K.12. Artificially inoculated panels appeared to be identical with naturally infected ones. Severity of disease on B.D.5 caused by form 2 from AVROS 50 was only slightly less than that on T.K.12 and it would be difficult to distinguish between them.

Similar inoculations were made in the wet season on clones B.D.10, B.D.5, and Tjir. 16 using 1 isolate of form 1, 7 isolates of form 2, 1 of which was obtained from leaves, and 1 isolate of *Pythium vexans.*

In general, the degree of infection on trees inoculated with *P. palmivora* was higher than had been obtained in the dry season; 50 to 85 per cent of trees inoculated with form 1 and about 100 per cent of those

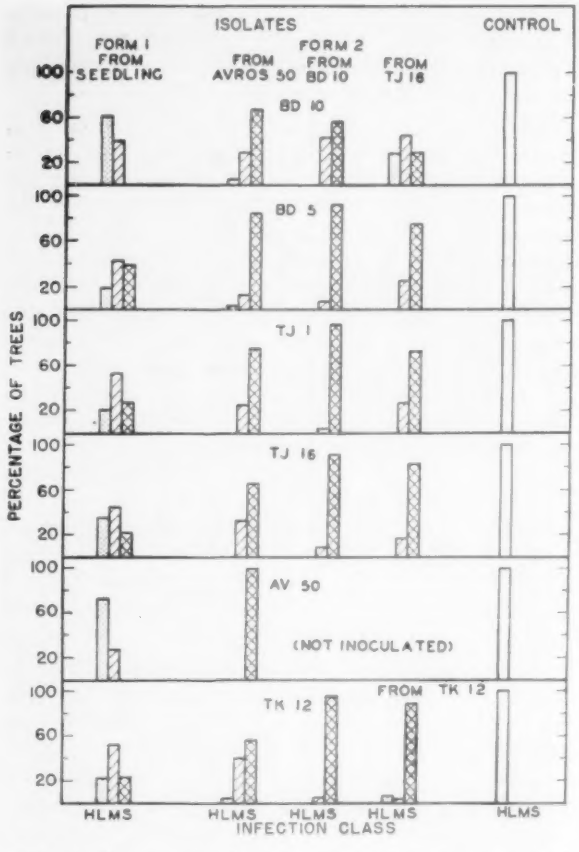


FIG. 5. Degree of infection, expressed as percentage of trees in 4 infection classes (healthy, light, medium, and severe), resulting from inoculating 6 clones of *H. brasiliensis* with various isolates of *P. palmivora* February-May 1946.

inoculated with form 2 had a medium to severe infection. For both forms, the percentage of infected panel appearing as patch lesions was greater than in dry season inoculations; with form 1 the figure was sometimes as high as 45 per cent while with form 2 it was usually 80 to 90 per cent. On trees inoculated with form 1, patches developed from coalesced streak lesions soon after inoculation. The isolate of form 2 obtained from leaves was as pathogenic as isolates obtained from panels.

No infection developed on any panels inoculated with *Pythium vexans*.

On Leaves and Non-wounded Stems.—An agar-mycelium mixture of each form of *P. palmivora* was smeared on the upper or lower surface of leaves, and also applied to leaf scars, base of petioles, and bark of the internode of very young trees in the greenhouse. Stems and leaves were covered with cotton and blotting paper moist chambers.

After 4 to 5 days large lesions appeared on the leaves inoculated on the lower surface, while those inoculated on the upper surface had few or no lesions. Lesions also were present at the site of leaf scars and at the base of the petioles. Infection occurred on the bark of the internode but developed later than on the leaf scars and petioles. The fungus was reisolated from the affected areas.

Results indicate that infection of twigs from which secondary leaf-fall has occurred takes place through leaf scars or even directly through the bark.

Progress of Disease and Recovery of Trees one year after Inoculation.—On some trees which, though heavily infected, often renew their bark to the point where there are no open wounds, the bark, may be gnarled and difficult to tap. Other trees with a slight infection may heal so that there is no evidence of disease in a year or two. On still others, the disease continues to develop, either downward with the tapping cut during the dry season or spread upward into previously healthy renewed bark.

On trees inoculated in February, 1946, and others inoculated in August, 1946, the degree of recovery or progress of the disease was observed for 1 year. Recovery was considered good when there were no open wounds, poor if there were a few open wounds, and none if more than 50 per cent of the inoculated area was still an open wound.

There was very good recovery of all trees inoculated with form 1 during the dry season of 1946 (Fig. 7, A). On trees inoculated with form 2, recovery varied from good to none and on some trees infection spread upward as much as 16 in. above the point of inoculation (Fig. 7, B, C, D).

In August, 1946, 550 trees of B.D.5 were inoculated with form 2 during one 15-day period. Severe infection developed on all trees. By April, 1947, the disease had spread upward on 149 or 27 per cent. On some, the spread was as high as 27 in. and all the bark on the full width of the panel was killed for most of that distance (Fig. 7, E).

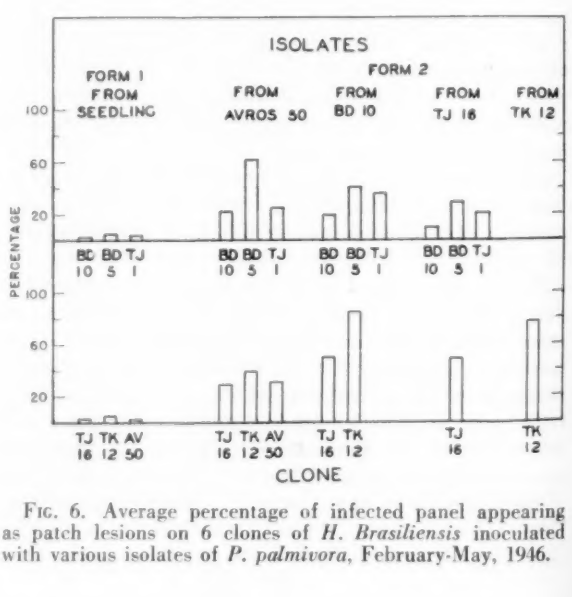


FIG. 6. Average percentage of infected panel appearing as patch lesions on 6 clones of *H. Brasiliensis* inoculated with various isolates of *P. palmivora*, February-May, 1946.

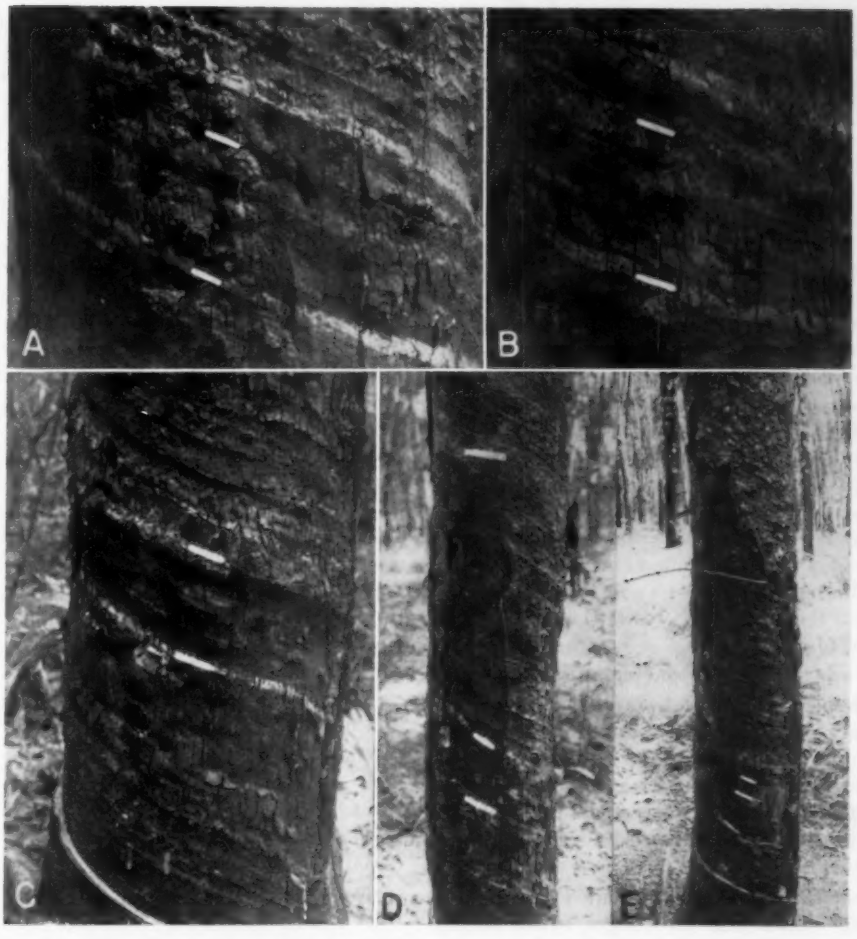


FIG. 7. Panels of B.D.5 one year after inoculation with *P. palmivora*. Panels in A to D inoculated in dry season on the 3 in. between marks. A) Inoculated with form 1. Shortly after inoculation infection rating was medium. Bark renewal was good and there was very little evidence of infection. B, C, and D) Three panels inoculated with form 2. Infection rating was medium, severe, and severe, respectively. There were still open wounds on B. In C there was no recovery and the disease spread downward with the tapping into the following wet season. In D the disease spread upward 16 in. into previously healthy renewed bark. E) Panel inoculated with form 2 in August 1946 on 1 in. between marks. Infection followed downward with the tapping into October. In April 1947 spread of infection upward 27 in. into healthy renewed bark became evident.

The disease reaction on these trees several months after inoculation was practically identical with that observed on naturally infected trees of seedlings and clones of B.D.5, AVROS 50, and T.K.12.

Effect of Tapping System on Infection.—Sharples, Belgrave, and others (12) have suggested that varying the tapping system from daily to alternate daily might reduce the incidence of disease. At Harbel, this was demonstrated on trees of clone B.D.5 where severe infection occurred on trees tapped half-spiral alternate daily while adjacent trees tapped full-spiral fourth daily were free from infection. Since the critical factor was probably the frequency of tapping rather than the length of cut, 3 tapping systems were used on inoculated trees to determine the effect of frequency. One plot each of B.D.5 was tapped on a half-spiral daily, alternate daily, and fourth daily. The daily plot was subdivided into 2 parts and each of the others into 3 parts, each part containing about 20 trees. All subdivisions were inoculated with form 2 for 15 days to establish the disease. Thereafter, for about 75 days, 1 part of each plot was inoculated daily, another part inoculated on the tapping day, and on the third part inoculation was discontinued.

Ninety-five to 100 per cent of the trees in all 3 tapping systems were severely infected when inoculated daily and on the tapping day. Where inoculation ceased after the first 15 days, infection continued to be severe with the daily and alternate daily systems but became progressively less with the fourth daily system until 37 per cent of the trees had light infection and 63 per cent medium infection. Thus it appears that once the disease is established, the fourth daily system retards further development of the disease provided there is no further severe inoculation. These results, coupled with field observations, suggest that full-spiral

fourth daily tapping offers a means of control of black thread. Spangler and McInode (13), however, could not recommend the system on most clones because of its detrimental effect on yield of latex and growth of trees.

Effect of Bark Renewal on Infection.—An experiment was made to determine the time interval after tapping during which the renewing bark remained susceptible to infection. In the dry season 40 trees of B.D.5 were tapped on the same day, a strip of bark 1 in. wide being removed in the 1 tapping. All panels were covered with moist bandages which were left on the trees for the duration of the experiment except when they were removed for a few minutes during inoculation. The panels were inoculated with form 2. Thirty-two of the trees were divided into 8 groups of 4 trees each. One group was inoculated on the day of tapping. Another group was inoculated 2 days after tapping and so on at 2-day intervals until the last group was inoculated 14 days after tapping. Thus the trees were allowed to renew their bark at intervals up to 14 days before being subject to infection. On the 8 control trees, the panels of 4 were smeared with sterile agar the day of tapping and nothing was put on the other 4 except the moist bandage.

Trees inoculated the day of tapping and 2 days after became severely infected. Trees inoculated 4 days after tapping had a medium to severe infection and those inoculated after 6 days had a very light infection. Bark regeneration had progressed sufficiently by the eighth day after tapping to resist infection completely.

Survival and Dissemination of the Pathogen.—McRae (8) has shown that the fungus exists during the dry season as mycelium near the junction of living and dead tissues on branches that have partially died back. Living mycelium also was found at the insertion of the stalk on fruits that had been infected the previous wet season. From these loci it became active at onset of the new wet season when rain falling through the leaves spread infection from dead branches to the new growth and to the fruit, leaves, and eventually to the panels.

In Liberia, *P. palmivora* was isolated during the wet season from the dead branches from which infected leaves had fallen, but as the dry season advanced isolation from such sources became infrequent. In 1 instance, the fungus was obtained from twigs in December. Isolations made later in the dry season were unsuccessful.

Older black thread wounds may be a site in which the fungus lives over dry periods as the fungus was isolated once from each of 3 wounds, 1, 2, and 3 years old.

In the laboratory, detached leaves were inoculated with a spore suspension and placed in a moist chamber. Other leaves, with pieces of young stem, were inoculated and buried 1 to 2 cm. deep in the soil. After 2 weeks, chlamydospores were found in the tissue of those leaves in the moist chamber. At the end of 60 days the fungus was still viable on the plant material buried in the soil, indicating that resting spores produced in the tissue of fallen leaves might also carry the fungus through dry periods or that the fungus would survive for some time on plant parts buried in the soil.

Sharples (12) observed that certain attacks of black thread seemed to be related to the path of the tapper and suggested that the tapping knife was an agent in dissemination. In an experiment to determine the role of the knife in spreading black thread in Liberia, 2 adjacent plots each 10 rows wide (east and west) and 16 rows long (north and south) were marked out. Trees in the 4 border rows on the north and south ends of the plots were tapped and inoculated with form 2 for 15 days to establish the disease. The 8 interior rows were not tapped or inoculated. For the following 15 days all trees in both plots were tapped; the worker walked north in the first row, south in the second, and so on. The next day the path of the worker was reversed for any given row. The trees were then rested 15 days and tapped again the following 15 days. In one plot the borders were inoculated every tapping day, while in the other, inoculation was discontinued. An adjacent block of trees tapped by a different worker, was not inoculated and served as a plot for observation of natural infection.

Ninety-one to 100 per cent of the trees in borders of both plots were severely infected and black thread spread to the interior trees. Infection was slightly higher in the plot where the border was under continual inoculation, with infection indices of 59 and 50, respectively. Averaging the 2 plots, the percentage of trees in the 4 infection classes of healthy, light, medium and severe, were 69, 8, 22 and 1 per cent, respectively. Natural infection in the control block was negligible and it was concluded that the disease in the interior rows of the inoculated plots was due to the carrying of inoculum on the knife from diseased to healthy trees.

The distribution of infected trees in the interior rows, with respect to their distance from inoculated borders was noted. The plots were not a solid planting as some trees were missing, so while each row should have had 4 healthy trees in from either border, there were usually 3 and occasionally only 2 which could be used in noting distribution. Most of the infection occurred on the first healthy trees tapped after tapping infected ones. Severity of infection on the second and third healthy trees decreased rapidly. Averaging the 2 plots, the percentage of trees in the 4 infection classes of healthy to severe were, respectively, for the first trees 47, 13, 38 and 2 per cent; for the second trees 82, 5, 13 and 0 per cent; and for the third trees 85, 5, 10 and 0 per cent. The infection indices for the first to third trees were 95, 31 and 25, respectively. The first tree had an infection index more than 3 times that of the second tree and almost 4 times that of the third tree.

It is evident that the disease can be spread by the

tapping knife. In this case, however, the spread was slow and at the end of 2 tapping periods, the disease was serious only on first trees tapped after tapping diseased ones.

CONTROL.—Many workers have reported various degrees of control of black thread with several fungisides. Since black thread is a wet season disease, one of the requirements of a fungicide is that it adhere to the panel and not be so diluted by rains after application as to become ineffective. Among the earliest recommendations (4, 8) was treatment of the cut with a mixture of tar and tallow, the tar serving as an antiseptic and the tallow as a waterproofing agent. From time to time proprietary tars and tar acid compounds were offered on the market. By 1936, 9 compounds were placed on an official "White List" by the Rubber Research Institute of Malaya as fungicides approved for the control of panel diseases (2). Those most commonly used against black thread were Izal, Brunolinum Plantarium, and Agrisol, representing the tar acid emulsions miscible with water, and Cargillineum mixture B., a fungicidal and waterproofing paste. Water miscible materials were applied to the panel daily or alternate daily at time of latex collection while the more permanent waterproofing pastes were used at intervals of several days.

In 1939, Beeley and Baptist (3) recommended palm oil as a diluent for fungicidal oils. In addition to acting as a carrier and sticker for the fungicides, the palm oil stimulated bark renewal.

Izal and Mouldos, proprietary fungicidal oils, mixed with palm oil, have been used with varying success in Liberia. In the last few years the relative ineffectiveness of the material against black thread on T.K.12 and the shortage and high cost of palm oil have made it necessary to search for new materials.

Preliminary Testing of Fungicides.—A number of fungicidal compounds were compared as to toxicity. In one series of tests the compounds were incorporated into agar and this was inoculated with a test fungus; in another series of tests sterile wood blocks were either impregnated or coated with fungicides at different concentrations and inoculated with test fungi. Relative efficacy was evaluated by observing degree of inhibition of the test fungi. These tests served to determine toxicity but did not permit evaluation of other important characteristics required of a fungicide on the plantations.

Testing of Fungicides on the Plantations.—a) *On naturally infected panels.* Based on results of preliminary tests, 3 fungicidal compounds were selected for testing on the plantations at Harbel: Laucks wound dressing 202-39 (pentachlorophenol), Phenyl mercury oleate, and Heyden Antiseptic Resin 173 (para hydroxy benzoic acid). Other fungicides tested included Dithane A 10 and Dithane D 14 (disodium ethylene bisdithiocarbamate), Isothan Q 61 (lauryl iso-quinolinium cyanide), Shirlan (salicylanilide), Socony Vacuum Products EF 487.2[6] (complex mixture of organic phenolic compounds derived from coal tar) and 2295-C (mixture of petroleum oil and waxy components without a fungicide), Chloramine B (sodium N-chloro benzenesulfonamide), and Noxtane (sodium pentochlorophenate). Dowax and Acrysol were used as stickers with some of the fungicides. Emulsion F, an emulsion of palm oil and Mouldos (tar distillate), which had been used in Liberia as the standard treatment for panel infection for several years, and the similar Emulsion J were also used.

Treatments extended over 2 to 4 tapping periods and the fungicides were applied daily or alternate daily at time of latex collection. The panels were not treated during the rest period. About 1 month after the last application, the effect of treatment was noted. Trees were placed into 2 arbitrary classes according to the degree of infection: (1) healthy to light, or less than 10 per cent of the panel infected, and (2) medium to severe, or more than 10 per cent of the panel infected. Results of using some of the fungicides on infected panels of T.K.12 and AVROS 50 during the wet season of 1945 and on AVROS 50 during 1946 are in Tables 1 and 2.

In 1945 Socony Vacuum Product EF 487.2 gave the best control. In addition, this material in this and subsequent tests visibly stimulated bark regeneration. Product 2295-C was less effective and not so easily applied. While natural infection on AVROS 50 was not so severe as on T.K.12, EF 487.2 and Emulsions F and J reduced infection.

Of the materials tested in 1946, Shirlan and EF 487.2 gave the best control. While the 20 per cent Shirlan in Dowax completely arrested further development of the disease, there was considerable difficulty in keeping the mixture from separating and constant

TABLE 1.—*Effect of several fungicides on natural black thread infection on clones T.K. 12 and AVROS 50 of Hevea brasiliensis, at Harbel, Liberia, August to November, 1945* [a]

Fungicide	Infection class and percentage of trees in each			
	Clone T.K. 12		Clone AVROS 50	
	Healthy to light	Medium to severe	Healthy to light	Medium to severe
Control	9	91	65	35
Emulsion F	45	55	92	8
Emulsion J	28	72	88	12
Socony Product				
EF 487.2	83	17	92	8
2295-C	68	32	75	25
Dithane A-10				
1 per cent in water	40	60	50	50
Isothan Q-61 in palm oil emulsion				
1:1250	15	85	24	76
1:2500	20	80	33	67
1:5000	20	80	52	48
Laucks wound dressing				
1:1 in water	4	96	61	39

[a] This test made by Dr. K. G. McIndoe.

[6] Compound EF 487.2 is now prepared under the trade name of Tree-Seal.

TABLE 2.—*Effect of several fungicides on natural black thread infection on clone AVROS 50 of Hevea brasiliensis at Harbel, Liberia, September to October, 1946*

Fungicide	Infection class and percentage of trees in each	
	Healthy to light	Medium to severe
Control	12	88
Socony Product EF 487.2	73	27
Emulsion F	12	88
Dithane 14 in water		
0.5 per cent	26	74
2.5	25	75
5.0	28	72
10.0	18	82
20.0	13	87
Dithane 14 in Dowax		
0.5 per cent	6	94
2.5	23	77
5.0	0	100
10.0	23	77
20.0	0	100
Antiseptic Resin 173	0	100[a]
Laucks wound dressing		
1:1 in water	23	77
2:1 in water	0	100[a]
Phenyl Mercury oleate in Dowax		
1 per cent	38	62
5	62	38
10	32	68
20	0	100[a]
Phenyl mercury oleate in Acrysol		
1 per cent	12	88
5	6	94
10	0	100
20	0	100[a]
Shirlan in Dowax		
1 per cent	42	58
5	54	46
10	67	33
20	100	0
Chloramine B in water		
1 per cent	20	80
5	0	100
10	0	100
20	0	100
Chloramine B in Dowax		
1 per cent	6	94
5	0	100
10	0	100

[a] Applied to healthy panels and found to be injurious to renewing bark.

supervision was necessary to insure the application of a homogeneous mixture to the panels. In addition, the cost of such a high concentration would probably be prohibitive. No control was obtained with Emulsion F.

Satisfactory control was not achieved with the 3 materials selected from preliminary tests. Moreover, they were injurious to the renewing bark.

For reasons of effectiveness, availability, and ease of application, EF 487.2 was used exclusively on the plantations during the 1947 wet season. In addition, further tests were made in which mixtures of Mouldos in EF 487.2 at rates of 0, 1, 3, and 5 per cent were applied alternate daily and twice weekly to plots of T.K.12 of about 60 trees each.

Initial natural infection prior to application of fungicides was not uniform on all plots so that a strict comparison between all treatments was not entirely legitimate. During treatment periods, however, natural infection on the several check plots developed about equally well. Treatments on plots with severe initial infection markedly reduced infection. Where infection was light, treatments prevented further development of disease. On the average, percentage of trees in the medium to severe infection class in untreated checks and treated plots was 84 and 17 per cent respectively. Results did not permit a definite discrimination between the effect of the various concentrations of Mouldos or frequency of application. Consequently application of EF 487.2 twice weekly would probably give as good control as any of the other combinations.

b) *On inoculated panels.* As noted above one difficulty encountered in testing fungicides has been the selection of areas where the disease was uniformly severe so as to allow for a comparison between treated and non-treated trees. Some tests have been discarded because the expected severe infection did not develop. A more satisfactory method would be to create an epidemic by artificial inoculation; then every tree would have a severe infection and more materials could be tested on fewer trees.

In July, 1947, trees of B.D.5 tapped on the alternate 15-day system were inoculated with form 2 of *P. palmivora* each tapping day for 1 tapping period to establish the disease. During the 2 following tapping periods fungicides were applied alternate daily but there was no further inoculation. Some inoculated trees were not treated and served as a control on further development of disease.

The mixtures of EF 487.2 and Mouldos were used in this test. In addition, Spergon (tetrachloro para benzoquinone) in a mixture with petroleum jelly, paraffin, and fuel oil[7] and Noxtane were also applied to the panels. The results are in Table 3.

The first trial of this new method of testing fungicides on inoculated trees was very satisfactory. A severe infection was obtained in the first tapping period. The disease continued to develop on control trees which received no treatment and thus afforded a satisfactory comparison with treated trees. All mixtures of EF 487.2 gave excellent control; there was no advantage gained in adding Mouldos. The Spergon paste was less effective and Noxtane gave no control.

DISCUSSION.—What appeared to be a new disease of the tapping panel on clone T.K.12 of *Hevea brasiliensis* on the Firestone Plantations in Liberia, is in reality a severe manifestation of the black thread disease caused by *Phytophthora palmivora* in which a prevalent and virulent strain of the fungus attacks a particularly susceptible clone of the host. Moreover, the severe form of the disease is not restricted to T.K.12 but is also found to some extent on clones

[7] This mixture suggested by Dr. W. J. Martin who used it for the control of Mouldy Rot disease of rubber in Mexico.

TABLE 3.—*Results of treating inoculated panels of clone B.D.5 of Hevea brasiliensis with fungicides. Panels tapped on alternate 15 day system; inoculated first tapping period with no fungicides applied, then treated the following 2 tapping periods with no inoculation*

Treatment	Infection class and percentage of trees in each	
	Healthy to light	Medium to severe
First tapping period: inoculated, no fungicides applied	0	100
Second and third tapping periods: fungicides applied, no further inoculation		
Control	7	93
EF 487.2 plus:		
0 per cent Mouldos	97	3
1 per cent Mouldos	97	3
3 per cent Mouldos	100	0
5 per cent Mouldos	100	0
Spergon paste	82	18
1 per cent Noxtane in Dowax	0	100

AVROS 50, B.D.5, B.D. 10, Tjir. 16, and on old seedling trees.

Of the 2 strains of *P. palmivora* found in Liberia, the so-called "atypical" strain, or form 2 of this study, is the more prevalent and virulent. It is able to produce all symptoms associated with black thread from the discrete, narrow, vertical lesions to the larger patch-like lesions which may involve the whole panel. Form 1 of the fungus is also pathogenic but regularly produces the narrow streak lesions. These results, coupled with isolation studies, suggest that most of the black thread in Liberia is caused by the atypical strain. The results also suggest that the similar severe variations of black thread reported from the eastern rubber growing countries, in which the bark is killed in large patches, may be the result of attack by the atypical strain of *P. palmivora*.

Of 3 possible control measures, i.e., use of resistant clones, changing the tapping system, or application of fungicides to the tapping cut, the last offers the best practical means of control. There is no indication at present that control can be assured through the use of resistant clones. All clones tested under artificial inoculation were classed as susceptible. B.D.5, B.D.10, and some other clones appeared to have some resistance under field conditions because in several cases they were not diseased while adjacent trees of T.K.12 had a severe infection. On the other hand, severe local epidemics sometimes occurred on these same clones. Trees under full-spiral fourth daily tapping were less susceptible to infection but the tapping system was detrimental to tree growth and yield of latex.

SUMMARY

Black thread, occurring principally in the wet season, is a disease of the tapping panel of rubber, *Hevea brasiliensis*, and is caused by *Phytophthora palmivora*.

What was thought to be a new panel disease was found in Liberia in 1940 on about 70 per cent of the trees of clone T.K.12. The disease was found subsequently on several other clones and on old seedling trees.

The studies reported herein show that the new disease is in reality a severe manifestation of black thread. In Liberia, there are at least 2 strains of *P. palmivora*, the so-called "typical' and "atypical" strains, designated in this study as form 1 and form 2, respectively. Form 2 is the more virulent strain causing, in addition to the narrow, vertical, streak lesions typical of black thread, the large patch-like lesions typical of infection on T.K.12. Form 1 regularly produces streak lesions, patch lesions only arising from the coalescence of several streaks. The prevalence of form 2 indicates that very little black thread in Liberia is caused by form 1.

Pythium vexans, cited as the cause of patch canker on rubber, was isolated from tapping panels but failed to cause infection when inoculated on panels.

Six clones were inoculated with various isolates of form 2. All clones were susceptible and all isolates about equally virulent. Under field conditions some of these clones, such as B.D.5 and B.D.10 appear to have some resistance, for at times they are free of disease while adjacent trees of T.K.12 are severely infected. However, severe local epidemics sometimes occur on these clones.

Unusual symptoms of black thread noted in Liberia included downward spread of infection during dry season tapping and upward spread into previously renewed and healthy bark. Both symptoms were produced on trees inoculated with form 2.

Infection was less severe on panels tapped fourth daily as compared to the daily or alternate daily tapping.

Renewing bark becomes resistant to infection within 4 to 6 days after tapping; after 8 days the bark is immune.

Black thread is spread by the tapping knife but not to a serious extent. In controlled experiments severe infection was found only on the first trees from an infection center after two 15-day tapping periods.

Control is best effected by fungicidal treatment. Several materials were screened in laboratory and field tests. The recommended treatment is Socony Vacuum Product EF 487.2 applied at least twice weekly. This material gave very good control and visibly stimulated bark renewal.

LITERATURE CITED

1. ASHBY, S. F. 1929. Strains and Taxonomy of Phytophthora palmivora Butler. Trans. Brit. Myc. Soc. **14:** 18-38.
2. BEELEY, F. 1936. Official grading of proprietary fungicides for treatment of Mouldy Rot of rubber trees. Jour. Rub. Res. Inst. of Malaya **7:** 8-19.
3. BEELEY, F., AND E. D. C. BAPTIST. 1939. Palm oil diluent for tar oil fungicides and its effect on bark renewal of Hevea. Jour. Rub. Res. Inst. of Malaya **9:** 40-50.

4. Dastur, J. F. 1916. Phytophthora sp. on Hevea brasiliensis. Mem. Dept. Agr. India, Bot. Ser. **8**: 217-232.
5. Ford, C. E. 1944. Experiments on the control of Bark Rot. Combined Quart. Circulars for 1944. Rubber Res. Scheme (Ceylon), p. 29-34.
6. Leonian, L. H. 1934. Identification of Phytophthora species. W. Va. Agr. Exp. Sta. Bul. 262.
7. McRae, W. 1918. A new species of Phytophthora parasitic on the Para rubber tree. Jour. Bombay Nat. Hist. Soc. **25**: 760.
8. McRae, W. 1918. Phytophthora meadii n. sp. on Hevea brasiliensis. Mem. Dept. Agr. India, Bot. Ser. **9**: 219-273.
9. Petch, T. 1921. Diseases and pests of the rubber tree. 278 pp. MacMillan and Company, Limited.
10. Sharples, A. 1917. Bark canker in Hevea brasiliensis. Kew Bul. Misc. Inform. No. 6, 219-225.
11. Sharples, A. 1936. Diseases and pests of the rubber tree. 480 pp. MacMillan and Company, Limited.
12. Sharples, A., W. N. C. Belgrave, and others. 1920. Black Stripe and Mouldy Rot of Hevea brasiliensis. Dept. Agr. Fed. Malay States Bul. 31.
13. Spangler, G. P., and K. G. McIndoe. 1949. Full-spiral tapping of Hevea brasiliensis. India Rubber World **119**: 592-595, 598, 723-724, 779.
14. Thompson, A. 1925. A preliminary note on a Phytophthora associated with patch canker of Hevea brasiliensis in Malaya. Malayan Agr. Jour. **13**: 139-141.
15. Thompson, A. 1929. Phytophthora species in Malaya. Malayan Agr. Jour. **17**: 53-100.
16. Tucker, C. M. 1931. Taxonomy of the genus Phytophthora deBary. Missouri Agr. Exp. Sta. Res. Bul. 153.

THE EFFECT OF SMOG ON THE ANATOMY OF OAT LEAVES [1]

Ruth Ann Bobrov [2]

At the present time it is known that smog[3] (6) in the Los Angeles area is damaging to various leafy crops, such as spinach, oats, endive, romaine, beets and sugar beets (5, 7, 12). Plant damage has been traced for 100 miles along the coast as far north as Oxnard and as far south as Oceanside. Streaking and tip burn of monocotyledonous plants have been reported by Zimmerman (13) in response to hydrofluoric acid and chlorine in the air under experimental conditions. Similar general symptoms, namely streaking and complete yellowing of newly matured leaves, have been found in oats (Avena sativa) in response to smog. This should not be construed to mean that the cellular damage induced by hydrofluoric acid and chlorine is identical with that induced by smog. The extent of smog damage depends upon its concentration; injury may be limited to a few leaves, but, in case of severe smog, the entire crop may be lost.

Immediately after the incidence of smog was reported by the Los Angeles County Air Pollution Control District, field crops were examined for incipient stages of damage. These examinations were followed by later periodic field observations. Plant response to smog has been observed in as little as 2 hours after the smog report was received.

Through the courtesy of Dr. Fritz Went and Dr. Herbert Hull of the California Institute of Technology, Pasadena, Kanota oat plants were grown smog-free in the specially filtered greenhouses of Earhart Laboratory. Experimental injury was induced in these plants by subjecting them to the oxidation products of unsaturated hydrocarbons obtained from ozone and gasoline vapors.[4] The injury induced in this way appeared, both to the eye and microscopically, similar to the damage obtained under field conditions.

Methods.—Microscopic examination was made of paradermal, transverse, and longitudinal hand sections of normal and smog damaged leaves. The amount of internal air space in the leaf is apparent in freshly cut hand sections which are immediately observed in water, but freezing and sectioning by means of a microtome altered cell shape and internal air spaces and led to plasmolysis of all cells. Hand sections of fresh material were therefore used in preference to embedded or frozen material. Excellent results were obtained by evacuating hand sections in glycerine, which replaced the air in the intercellular spaces. No apparent change in size of cells or intercellular spaces was induced by this technique. Such sections could later be stained with thionine, or other desired dyes, and were suitable for photomicrography, since the air pockets, normally troublesome, were removed and the stain was taken up evenly by cell walls. Such slides were kept for as long as a week with very little alteration.

Detection of anatomical damage from smog was enhanced by staining sections in various ways. Damaged cells stained readily with Sudan III and Sudan black and, in transverse and paradermal sections, stood out clearly from normal cells.

The indicator stain thionine (in aqueous solution)

[1] Accepted for publication June 23, 1952.

[2] Research Botanist Air Pollution Control District, County of Los Angeles.

[3] Smog is a complex mixture of liquids, solids and gases, comprising more than 50 chemical elements and compounds and producing, among other effects, low visibility, eye irritation, crop damage, excessive rubber cracking and odor nuisances.

Grateful acknowledgment is made for the generous assistance of Dr. F. M. Scott and Miss Elizabeth Baker of the Botany Department, University of California at Los Angeles. Thanks ars also due Mr. Wilfred Noble of the Los Angeles County Air Pollution Control District for kindly furnishing materials whenever needed.

[4] Two tenths of a part per million of ozone mixed with gasoline in large enough quantity to react completely with the gasoline. Caution was necessary so that no free ozone, which could be damaging, was delivered to the plants. A description of the procedure may be found in a paper by Haagen-Smit et al (2).

is a very useful general stain, especially where material for photomicrography is desired. It was taken up readily by cell walls and served to delimit the xylem of the vascular bundles, staining the lignin of the xylem elements blue and the parenchymatous non-lignified cell walls purple.

Phloroglucin followed by hydrochloric acid indicates the extent of lignification of the leaf. Some of the sclerenchyma cells of oat are quite resistant to this lignin test.

The general pattern of venation was studied by clearing leaves in chloral hydrate and staining with phloroglucin and hydrochloric acid.

IKI and sulfuric acid were used to indicate the comparative thickness of cuticle on lower and upper epidermis. They also revealed internal suberization of cells (8).

Observations of normal leaf.—In Los Angeles in February the oat crop stands about 15 to 20 cm. tall. When such plants are affected by smog, the youngest and oldest leaves remain virtually undamaged, while newly matured leaves are hardest hit; those leaves that have just completed their growth in area by cell expansion seem most susceptible.

In its anatomy the oat differs from wheat and corn in various respects (3, 4). In shape, the oat leaf is that of a typical grass with sheathing base, ligule, and strap-shaped, parallel-veined blade. The upper surface of the leaf is rough, due to the prominence of the veins, while the lower surface is comparatively smooth.

Tissues of the oat leaf include upper and lower epidermis, mesophyll and vascular elements. As in many grasses, cells of the epidermis are not homogeneous in size; their shape, in general, is rectangular. Cells of the vascular ridges are flanked by those of the stomatal zones. Adjoining stomatal zones are separated by cells of the motor or bulliform zones. In transection, therefore, the following normal anatomical zones may be distinguished (Fig. 1): (a) a zone of vascular elements which will be designated the vascular ridge, (b) a zone of loosely arranged armed cells, with stomata on both epidermal surfaces, which will be called the stomatal zone, and (c) a zone of more compact, smaller and less lobed cells, with bulliform cells only on the upper epidermis, which will be known as the motor zone. The pattern repeats itself throughout the width of the leaf: vascular ridge, stomatal zone, motor zone, stomatal zone, vascular ridge, stomatal zone, motor zone, etc.

The epidermis is composed of rectangular cells parallel to one another and elongated in the long direction of the blade. The stomata pierce the epidermis and extend in parallel longitudinal rows from the tip to the base of the leaf. They are confined to the stomatal zones. On the adaxial surface, there are 3 longitudinal parallel rows of epidermal cells in each stomatal zone, the stomata being staggered in these rows (Fig. 2A). The chlorenchyma cells below the stomata are loosely packed. In longitudinal section, the tremendous amount of internal air space may be seen (Fig. 2B); for this reason, damage by smog is most severe in the stomatal regions. On the vascular ridges, unicellular, awl-shaped trichomes arise regularly and in single longitudinal file from an epidermal cell on the upper and lower surface of the leaf.

On the adaxial surface also, 3 or 4 of the epidermal cells are modified on either side of the stomatal cells into bulliform or motor cells. Such cells are completely lacking on the lower surface. Bulliform cells are less cutinized than other epidermal cells. They are of use to the plant during drought when they lose water to the atmosphere and constrict, causing the lamina to curl, thus protecting the leaf from desiccation by reducing the amount of transpiring surface. The bulliform cells are less than half as long, 1½

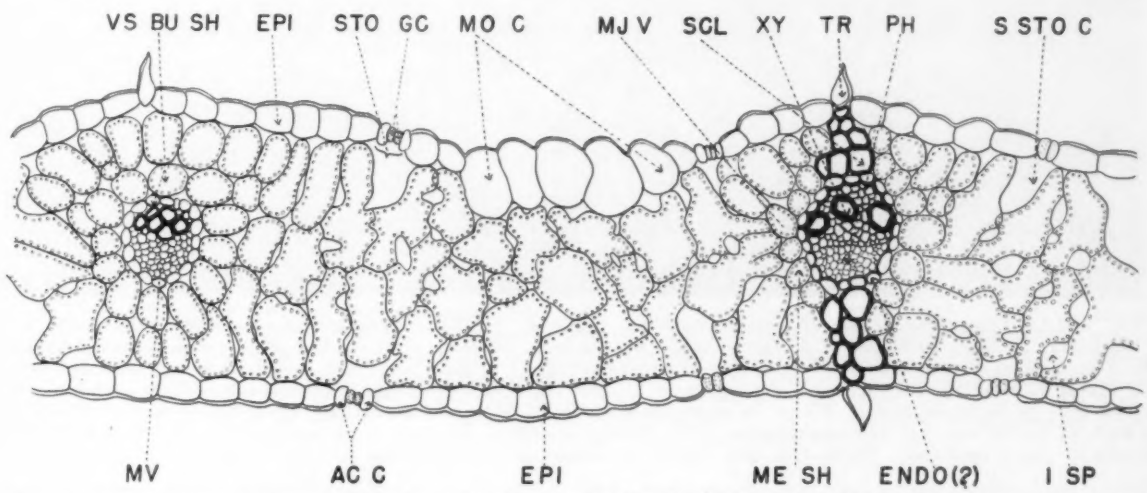


Fig. 1. Cross section of normal oat leaf; vs bu sh, vascular bundle sheath; epi, epidermis; sto, stomata; g c, guard cell; mo c, motor or bulliform cells; mj v, major vein; scl, sclerenchyma; xy, xylem; tr, trichome; ph, phloem; s sto c, substomatal chamber; mv, minor vein; ac c, accessory cell; me sh, mestone sheath; endo, endodermis; i sp, intercellular space.

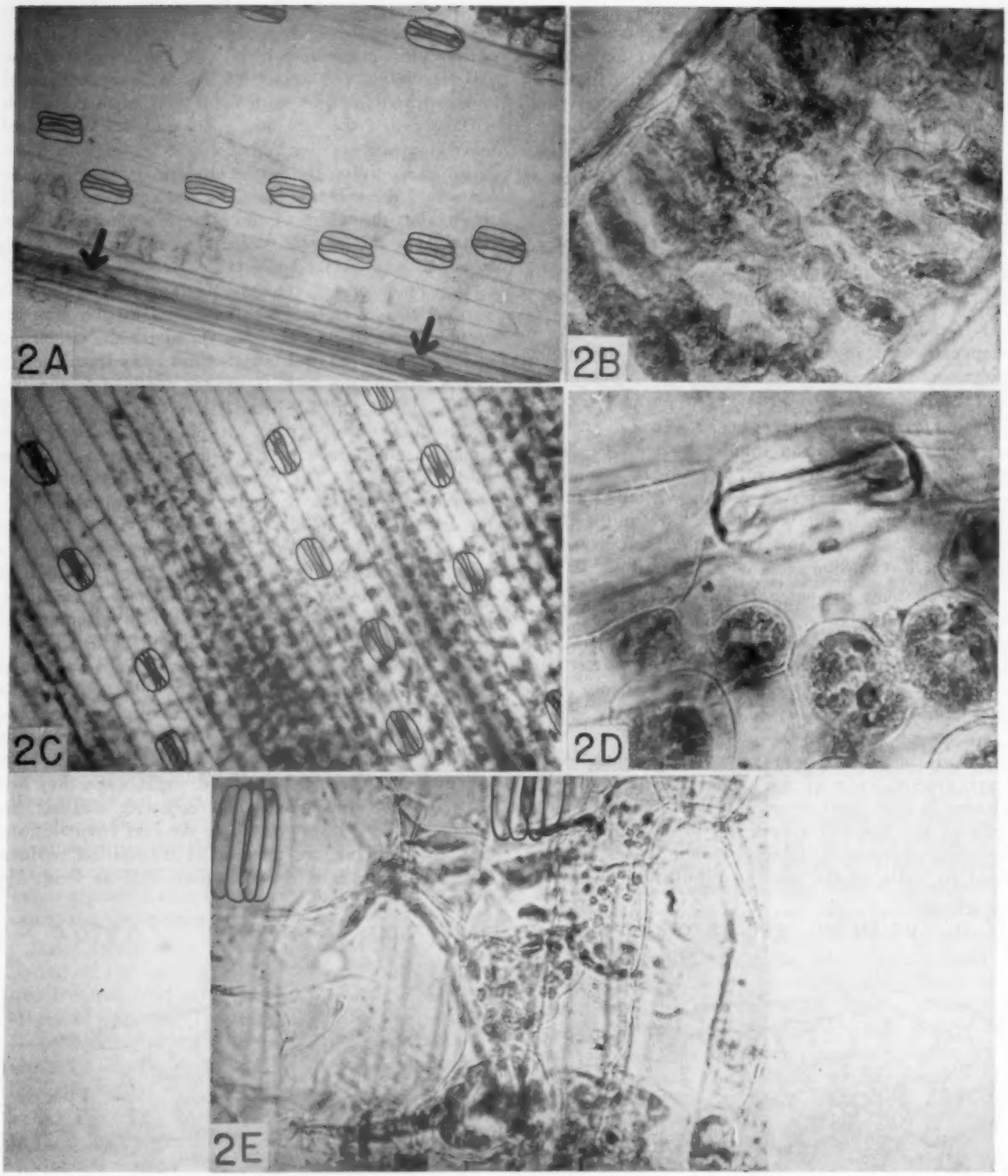


FIG. 2. (A) Linear distribution of stomata on upper epidermis (×100). Approximate size of epidermal cells, 650 × 35 micra; bulliform cells, 280 × 43 micra; stomatal cells 65 × 35 micra. Arrows point to trichomes on vascular ridge. (B) Longitudinal section through stomatal zone of undamaged leaf (×215). Note large amount of intercellular air space. (C) Linear distribution of stomata on lower epidermis (×100). Approximate size of epidermal cells, 443 × 29 micra; epidermal cells in bulliform region, 700 × 29 micra; stomatal cells, 65 × 35 micra. (D) Substomatal chamber delimited by walls of highly branched chlorenchymatous cells. Normal (×645). Incipient smog damage in cells directly beneath stomata of upper epidermis. Plasmolysis and stages in collapse of cell walls may be seen (×645).

times as wide, and deeper than the epidermal cells in other regions. There are no stomata present in this region.

On the lower epidermis there are no bulliform cells. The stomatal cells here are fewer and farther separated from one another than on the upper epidermis

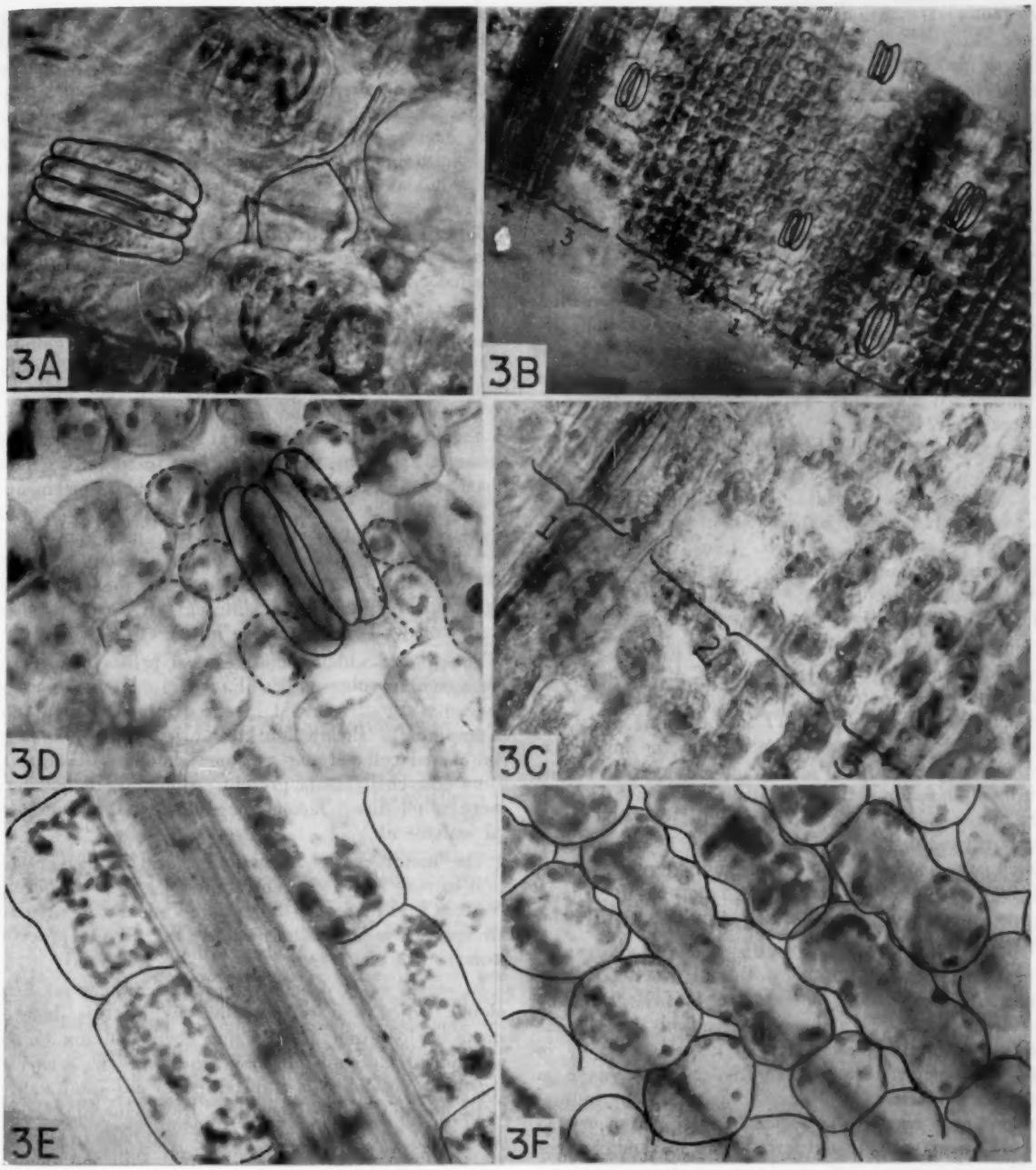


FIG. 3. (A) "Mummification" of smog damaged cells directly below stomate (×645). (B) Pattern of damage in paradermal section of leaf. (×100). Zones 1 and 3 are stomatal zones in which cells are dead and shrunken causing streaking. Zones 4 are vascular ridges. Zone 2 is motor zone. (C) Paradermal section of normal leaf (×215). 1) Minor vein with brick-shaped mestome sheath cells. 2) Stomatal zone with highly branched cells and large intercellular space. 3) Compact cells of motor zone. (D) Paradermal section through cells of stomatal region. Normal (×430). Note narrow arms seen in transverse section (dotted lines) of large branched cells. Note, also, extent of intercellular space. Fig. 9, zone 2 enlarged). (E) Mestome sheath cells with chloroplasts surrounding minor vein. Paradermal view. Normal (×430). Cells of inner sheath not in focus. (Fig. 9, zone 1 enlarged). (F) Paradermal section through motor zone. Normal (×430). Note compact nature of chlorenchymatous cells and intercellular air space.

and are only in 1, or at most 2, parallel series in each stomatal zone. The distance between the stomata on this surface is almost twice as great as the distance between the stomata on the upper epidermis (Fig. 2C). Epidermal cells in the motor region on the lower surface are long and narrow, some measuring almost 1 ml. in length.

Beneath each stomate is a substomatal chamber of

substantial size. Cells which line the chamber are large, eight-armed, (9, 11) and expose much wall surface to air space (Fig. 2D). The chlorenchyma cells in the motor zone are smaller than those in the stomatal zones but are also armed. They are more compact and possess less intercellular space than do the cells of the stomatal zones.

To the unaided eye, the leaf appears ridged above and almost smooth below. Ridging is due to the parallel longitudinal arrangement of vascular bundles as they alternate with the motor region tissue. Cells of the motor region are more compactly arranged than any other tissue in the leaf so that, in transection, the motor region appears constricted between veins. Every third vein is a major vein, which is large and well strengthened above and below by thick walled sclerenchyma cells. Between the major veins there are 2 minor veins which are not at all or only meagerly supported by sclerenchyma. Occasionally a single strand or 2 of sclerenchyma is found just under the upper and lower epidermis. Small connecting veins run transversely between the parallel veins. Sclerenchyma thickening in connection with veins, together with the constricted nature of the motor regions, results in visible ridging of the leaf.

Vascular bundles are collateral and phloem is present except in some transverse bundles. A double sheath surrounds the bundles with the internal layer an endodermal-like inner sheath (1). In the major veins, the cell walls of this layer are heavier on the inner tangential and lateral than on the outer tangential walls, while in the minor veins, all walls are evenly thickened. The outer sheath or mestome sheath is made up of fairly thin-walled, brick-shaped, parenchymatous cells which are elongated in the longitudinal plane. Cells of the mestome sheath contain chlorophyll. The 2 layers comprising the vascular bundle sheath completely encircle the bundle.

Observations of smog damaged leaves.—Smog damage to cells of crop plants was first described by Middleton et al (7) and more extensively by Thomas (10). No specific description of cellular damage in oats has been reported previously. Smog damage in oats first manifests itself in the field as longitudinal streaking of leaves of affected plants. After an exposure of a few hours, oat leaves show marked changes in the substomatal regions. Incipient damage can be clearly demonstrated to originate in the cells directly below the stomata even before yellowing becomes apparent to the eye (Fig. 2E). Soon after fumigation, in the cells which directly line the substomatal chambers, the chloroplasts begin to show alteration in shape prior to their complete disorganization. Plasmolysis occurs, the protoplasmic contents of the affected cells shrinking to the interior. Plasmodesmatal connections with neighboring cells are maintained; however, the cell walls then collapse, resulting in dehydration and "mummification," a characteristic of smog-damaged cells (Fig. 3A), (6). Low concentrations of toxic materials in the air cause limited damage which is not lethal to the plant; higher concentrations cause more extensive damage involving several cells on either side and below or above the primarily attacked cells; very high concentrations may involve all the cells of the leaf, causing complete death. Early damage shows as longitudinal streaking corresponding in position to the stomatal zones, while later damage due to continued exposure manifests itself as complete yellowing and death of the entire blade. Fig. 3B illustrates the pattern of damage in paradermal section of a mildly affected oat leaf.

Discussion.—The type of cellular injury seen in smog-exposed oat leaves is determined by the anatomical structure of the normal leaf (Fig. 3C). Injury depends upon 2 anatomical factors: (a) the presence of stomata and (b) the amount of internal air space in the leaf. While the oat leaf is a highly ventilated system throughout, the stomata are restricted in their distribution to those areas that possess a great amount of internal air space. Stomatal areas are the most severely damaged by smog, since here both anatomical factors favor damage; the stomata present a portal of entry for the noxious elements from the outside air and the large amount of internal air space exposes many cell walls for extensive injury (Fig. 3D).

The region which almost always resists damage is the vascular ridge (Fig. 3E). Here the conducting elements are surrounded by the lignified cells of the inner bundle sheath and the brick-shaped, densely compacted cells of the mestome sheath. In addition, some sclerenchyma is present. Stomata are lacking; there is no direct means for diffusion of gases from the outside air.

The motor region likewise is rarely damaged except in very heavy concentrations of smog, in spite of the fact that there is a fairly large amount of internal air space present in this region (Fig. 3F). However, stomata are absent.

In severe smog concentrations, a leaf may be completely killed and turn yellow. While this damage starts as longitudinal streaking corresponding to the position of the stomatal zones, these streaks soon run together as damage is transmitted to adjoining zones. Cells are not isolated entities. By means of plasmodesmatal connections, the large armed cells of the stomatal zones are in intimate contact with the smaller ones of the motor zones and with the brick-shaped ones of the vascular bundle sheaths. When the concentration of smog in the air is high, and many cells in the stomatal zones are killed, the remaining tissues are eventually destroyed and the leaf dies.

SUMMARY

Smog in the Los Angeles area causes serious damage to the oat crop. Damage depends upon the normal anatomical pattern of the parallel-veined leaf, upon the stomata as a portal of entry for the harmful elements in the air, and upon the amount of internal air

space in the leaf. Initial damage may be observed microscopically as the cells directly lining the substomatal chambers shrink. As more and more cells are affected, longitudinal streaking of the leaves occurs, corresponding to the position of the stomatal zones. Ultimate browning and death of the entire blade may result. The extent of injury is in proportion to the concentration of smog in the air.

LOS ANGELES COUNTY AIR POLLUTION CONTROL DISTRICT
LOS ANGELES, CALIFORNIA
AND
DEPARTMENT OF BOTANY
UNIVERSITY OF CALIFORNIA
LOS ANGELES, CALIFORNIA

LITERATURE CITED

1. EAMES, A. J. AND L. H. MACDANIELS. 1947. An introduction to plant anatomy, McGraw-Hill, New York and London, 427 pp., 2nd. ed.
2. HAAGEN-SMIT, A. J., E. F. DARLEY, M. ZAITLIN, H. HULL AND W. NOBLE. 1952. Investigation on injury to plants from air pollution in the Los Angeles area, Plant Physiol. **27**: 18-34.
3. HAYWARD, H. E. 1948. The structure of economic plants. The McMillan Co., New York, 674 pp.
4. HECTOR, J. M. Introduction to the botany of field crops, Vol. I—Cereals, Central News Agency, Ltd., Johannesburg, So. Africa, 478 pp.
5. LOS ANGELES COUNTY AIR POLLUTION CONTROL DISTRICT. 1949/50. Technical and administrative report on air pollution control in L. A. County Ann. Rep., 49 pp.
6. LOS ANGELES COUNTY AIR POLLUTION CONTROL DISTRICT. 1950/51. Technical and administrative report on air pollution control in L. A. County Ann. Rep., 52 pp.
7. MIDDLETON, J. T., J. B. KENDRICK, JR., AND H. W. SCHWALM. 1950. Injury to herbaceous plants by smog or air pollution. U.S. Dept. Agr. Plant Dis. Rep. **34**: 245-252.
8. SCOTT, F. M. Internal suberization of tissues. 1950. Bot. Gaz. **111**: 378-394.
9. SCOTT, F. M., M. R. SCHROEDER AND F. M. TURRELL. 1948. Development, cell shape, suberization of internal surface and abscission in the leaf of the Valencia orange, Citrus sinensis. Bot. Gaz. **109**: 381-411.
10. THOMAS, M. D. Gas damage to plants. 1951. Ann. Rev. of Plant Physiol. **2**: 293-322.
11. TURRELL, F. M. 1936. The area of the internal exposed surface of dicotyledon leaves. Amer. Jour. Bot. **23**: 255-264.
12. WENT, F. W. 1950. The general problem of air pollution and plants. Proc. First National Air Pollution Symposium, pp. 148-149. Stanford Research Institute, Los Angeles, California.
13. ZIMMERMAN, P. W. 1950 Impurities in the air and their influence on plant life. Proc. First National Air Pollution Symposium, pp. 135-141. Stanford Research Institute, Los Angeles, California.

ALTERING RESISTANCE TO DISEASE WITH SYNTHETIC ORGANIC CHEMICALS [1]

David Davis [2] and A. E. Dimond

Two approaches have been emphasized in plant chemotherapy investigations. These include the search for (a) systemic fungicides as exemplified by the synthetic organic toxicants (7, 10, 17) and antibiotics (3, 4, 5, 18) and (b) anti-toxins (9, 12, 13).

Early in the development of the chemotherapy program at The Connecticut Agricultural Experiment Station, Horsfall and Dimond (11) noted that there was not necessarily a direct correlation between fungitoxicity to *Stemphylium sarciniforme* (Cav.) Wiltshire spores and chemotherapeutic activity on the Fusarium wilt disease of tomato. Many compounds, although poorly fungitoxic, were excellent chemotherapeutants. However, this comparative study was limited by the use of 2 different organisms and because the inhibition of spore germination was being compared to the inhibition of mycelial growth. Accordingly, investigations were undertaken to (a) make the comparisons more applicable and (b) study the mode of action of a nonfungitoxic and a fungitoxic chemotherapeutant.

EXPERIMENTAL.—Fungitoxicity determinations were performed on the mycelium of *Fusarium oxysporum* f. *lycopersici* (Sacc.) S. & H. The fungus was incubated at room temperature (approximately 20° C.) for 3 days in a casamino acid nutrient broth. The mycelium was then harvested, ground in a Waring blendor for ½ minute, washed twice with M/60 phosphate buffer, pH 6.2, and resuspended in the phosphate buffer. The concentration of macerate was standardized with a Fisher electrophotometer. This suspension served as inoculum for growth determinations and was used in respiratory studies.

Since certain ingredients of a nutrient medium have been shown to suppress fungitoxic activity (6, 26), it was considered desirable to test the fungitoxicity of potential chemotherapeutants in the simplest well defined medium capable of supporting growth or respiration. Fortunately, *F. oxysporum* f. *lycopersici* is a highly autotrophic fungus. Richard's nutrient (19),

[1] Accepted for publication June 27, 1952.
This study was conducted in cooperation with the Crop Protection Institute. We wish to express our gratitude to Miss Barbara Wooding for her technical assistance. The authors wish to acknowledge the help of Dr. Alfred N. Meiss, Dept. of Biochemistry, The Conn. Agr. Expt. Sta. who made the soluble nitrogen determinations and offered some helpful suggestions. We also wish to thank Mr. L. G. Keirstead and Mr. A. F. Wichroski of the Dept. of Analytical Chemistry, The Conn. Agr. Exp. Sta. who made the reducing sugar determinations. Rohm & Haas Co., Washington Square, Philadelphia, Pa., and Carbide and Carbon Chemicals Division, Union Carbide and Carbon Corp., 30 E. 42nd St., New York, N. Y., supplied some of the compounds used in these studies.
[2] Plant Pathologist, Crop Protection Institute.

TABLE 1.—*Comparison of the chemotherapeutic activity of various compounds on the Fusarium wilt disease of tomato with their fungitoxicity to F. oxysporum f. lycopersici*

Compound	Chemotherapeutic activity		Fungitoxicity		
	Conc. ppm	Per cent decrease in vascular discoloration	Conc. ppm	Mycelial growth	Per cent inhibition Oxygen uptake[a]
Sodium 2-benzothiazolyl thio-glycolate[d] (HD 160)[b]	7.8	39	25	0	0
	15.6	64	250	24	0
	62.5	82	1250	73	0
2-(n-amyl) pyridine (HD 139)[b]	7.8	40	15	19	0
	15.6	44	150	19	0
	62.5	72	750	26	0
4-chloro-3,5-dimethylphenoxyethanol (1182)[c]	3.9	62	2	3	
	7.6	84	20	21	0
	15.6	96	200	100	
			1000	100	
n-octadecyl-trimethylammonium pentachlorophenate (HD 3)[b]	16.6	62	0.6	17	
	25	86	6	55	
	37	100	60	100	100
8-hydroxyquinoline benzoate	500	0	2.5	100	
			25	100	25
			250	100	25

[a] Measured during the 6th hour after application of the compound.
[b] Supplied by Rohm & Haas Co., Philadelphia, Pa.
[c] Supplied by Carbide and Carbon Chemicals Division, Union Carbide and Carbon Corp., New York, N. Y.
[d] Synonymous with 2-carboxymethylmercaptobenothiazole, a name used by the authors in previous publications in this journal.

an inorganic salt, glucose medium could therefore be used to grow the mycelium. The nutrient medium containing the test compounds was adjusted to pH 6.2, autoclaved for 15 minutes at 15 lbs. pressure and inoculated with ½ ml. of the previously described mycelial suspension. The dry weight of the mycelium was determined 10 days after inoculation.

Inhibition of oxygen uptake of endogenously respiring mycelium was used as another measure of fungitoxicity. Since the assay was made with a medium devoid of all nutrient except phosphate buffer, the chances of masking the inherent fungitoxicity of the compound being tested was further minimized. Typical Warburg techniques were used in the assay. Upon equilibration, the compounds under test were added to the chamber containing the mycelial suspension. Oxygen uptake was measured at fixed intervals. The assay was performed at 29° C.

The Bonny Best variety of tomato was used in all tests. Unless otherwise stated, chemotherapeutic activity was measured by the method of Dimond et al (8).

A comparison of the chemotherapeutic activity of a number of compounds against the Fusarium wilt disease with their fungitoxicity to the pathogen indicated no consistent relationship (Table 1). The 10 test compounds fell into 3 categories: (a) excellent chemotherapeutants but poor fungitoxicants, (b) excellent chemotherapeutants and good fungitoxicants, (c) poor chemotherapeutants but excellent fungitoxicants. Thus, sodium 2-benzothiazolyl thioglycolate (HD 160) and 2-(n-amyl) pyridine (HD 139) are excellent chemotherapeutants but poor fungitoxicants; 4-chloro-3,5-dimethylphenoxyethanol (1182) and n-octadecyltrimethylammonium pentachlorophenate (HD 3) are both good fungitoxicants and chemotherapeutants; 8-hydroxyquinoline benzoate is a poor chemotherapeutant yet has remarkable fungitoxic activity. Except for 8-hydroxyquinoline benzoate, the inhibition of mycelial growth was well correlated with endogenous respiration.

HD 160 and 1182, representing 2 types of chemotherapeutants, were chosen for investigations on their modes of action. HD 160 is a poor fungitoxicant while 1182 is a good fungitoxicant (Table 1). Conceivably an interaction of HD 160 and the biochemically active plant might result in the production of a fungitoxic moiety. Accordingly, experiments were designed to test for the presence of a fungitoxic component in the tissue of plants treated with these chemotherapeutants. Tomato plants in the 6-leaf stage were placed, with roots immersed, in 100 ml. of 15.6 ppm 1182 and 62.5 ppm HD 160 for 6 days. After treatment the plants were harvested, ground in a mortar, diluted 4-fold with a 4 per cent agar solution and poured into petri dishes. One aliquot of plant material was sterilized by autoclaving for 10 minutes at 10 lbs. pressure. Another aliquot was treated with 10 ppm of methylene blue to reduce bacterial contamination. The agar-macerate was then inoculated with 0.1 ml. of a washed 4-day-old shake culture of *F. oxysporum* f. *lycopersici* bud cells. The inoculum was placed in the center of a petri dish

and the radial growth of the fungus was measured after 72 hours.

The data in Table 2 demonstrate that the fungus grew equally well on the macerate of both treated and untreated plants. Thus no fungitoxic moiety was detected in plants treated with either 1182 or HD 160.

TABLE 2.—*The growth of F. oxysporum f. lycopersici on the tissue macerate of plants treated with 15.6 ppm 1182 and 62.5 ppm HD 160*

Treatment	Millimeters of growth at 72 hours	
	Methylene blue sterilization	Heat sterilization
1182	8.0	9.5
HD 160	8.5	9.5
Check	8.5	9.5

Since *in vitro* studies (Table 1) have shown 1182 to possess good fungitoxic properties, it is possible that if this material were concentrated in the vascular system of the host plant, it would exert a deleterious effect upon the invading fungus. To test this point, tomato plants were treated with 15.6 ppm 1182. At the completion of the treatment period, the stem was cut 1 in. above the crown and the sap exuding due to root pressure was collected. It was then assayed for fungitoxicity to *S. sarciniforme* by the method of Barratt and Horsfall (2).

A 2-fold dilution of the liquid exudate from plants treated with 1182 was not fungitoxic. By interpolation from the data in Table 3 it is evident that the exudate contained less than 5 ppm 1182. As shown in Table 1 this concentration is innocuous to *F. oxysporum* f. *lycopersici*.

TABLE 3.—*The fungitoxicity of 1182 to S. sarciniforme*

Compound	Per cent inhibition of spore germination		
	50 ppm	5 ppm	0.5 ppm
1182	100	48	0

Again, the chemotherapeutic properties of 1182 could not be attributed to its direct fungitoxic action on the parasite.

Although a fungitoxic component could not be detected in the tissue of tomato plants treated with either HD 160 of 1182, these compounds induced both morphological and biochemical modifications of the host. Both compounds produced a formative effect on the leaves. 1182 and HD 160 induced leaf modifications resembling 2,4-D and α-naphthalene-acetic acid respectively. Whereas 1182, at times, affected the new growth after treatment had ceased, HD 160 did not. HD 160 treated plants were also characterized by a rosetting of the leaves which had developed during the treatment period. The fact that 1182 and HD 160 produced formative effects is considered strong evidence that these compounds entered aerial portions of the plant.

Could the chemotherapeutic activity of HD 160 and 1182 be attributed to the accumulation of a fungitoxic concentration of these chemicals on or in the roots? These possibilities are considered unlikely. The tomato wilt organism enters the plant primarily through injured roots. In the chemotherapy assay the roots are washed and the lower portions torn off just prior to inoculation. Thus, even if there were an accumulation of the chemical on the root surface it would not be likely to operate in reducing disease. The evidence also indicates that a fungitoxic concentration of 1182 and HD 160 did not accumulate within the roots.

In making up the agar-macerate mixtures from plants receiving 62.5 ppm HD 160 and 15.6 ppm 1182, the root macerate was diluted 16 times. The data in table 1 indicate, by interpolation, that a 1/16 dilution still has considerable chemotherapeutic activity. The agar-macerate mixture, however, was not inhibitory to the mycelial growth of the parasite (Table 2). These facts argue against the possibility that the chemotherapeutic effect was caused by the accumulation of a fungitoxic component within the roots.

In addition to the observed morphological modifications 1182 and HD 160 induced a number of biochemical changes in the tomato plant. Five tomato plants in the 6-leaf stage were placed, with roots immersed, in 100 ml. of a 15.6 ppm 1182 or 62.5 ppm HD 160. After 6 days the treated plants were harvested and macerated in a Waring blendor for 2 minutes. One portion of the tissue macerate was used in respiratory studies performed by the usual Warburg techniques. Another was filtered and assayed for reducing sugar content by A.O.A.C. methods (1) and water soluble nitrogen fractions as prescribed by Vickery et al (23). A number of quantitative changes in these components were observed.

1182 treated plants showed an increase in reducing sugar content, in all but 1 (asparagine) of the water soluble nitrogen fractions, and in rate of oxygen uptake. HD 160 increased the concentration of all the water soluble nitrogen fractions but did not affect the reducing sugar content. Respiratory studies with HD 160 were inconclusive (Table 4).

These studies suggest that 1182 and HD 160 increased resistance of the tomato to the Fusarium wilt disease by producing changes in the metabolism of the host unfavorable for development of disease. Furthermore, since the parasite grew equally well on the tissue of treated and untreated plants (Table 3), it would appear that the inhibition of the fungus within the host is not due to a modification of the nutritive level or a metabolically produced fungitoxic component.

DISCUSSION AND CONCLUSIONS.—Experimental evidence has been presented which indicates that there is not necessarily a direct relation between fungitoxicity of compounds and their chemotherapeutic potency. Even when compounds are moderately fungitoxic, apparently they need not enter the plant in fungitoxic concentrations to be active as chemotherapeutants. As

TABLE 4.—*The effect of 1182 and HD 160 treatment on the reducing sugar content, water soluble nitrogen fractions and oxygen uptake of tomato plants*

	Milligrams per gram fresh weight					μL per hour per gram fresh weight[a]
Treatment	Free NH_3	Glutamine N	Asparagine N	α-Amino N (Free)	Reducing sugar expressed as invert sugar	oxygen uptake
1182	0.064	0.192	0.108	0.340	10.3	800
HD 160	0.061	0.182	0.164	0.367	7.73	.....
Check	0.044	0.096	0.108	0.206	7.78	631

[a] Oxygen uptake during the 7th hour of the assay.

an example, 1182 was not present in the plant in fungitoxic concentrations although it reduced the severity of Fusarium wilt appreciably. HD 160 reduced the severity of disease at concentrations which had no effect upon the growth of Fusarium.

When these compounds were applied to plants, the reducing sugar level and rate of oxygen uptake were increased by 1182 while the water soluble nitrogen components were higher in plants treated either with 1182 or HD 160. The metabolism of the treated plants was evidently altered.

The susceptibility of plants to disease may be influenced by such shifts in metabolism. Apparently the kinds of changes in nutritional levels that bring about an altered resistance to disease are not at all specific. By simply altering the level and balance of nutrient elements supplied the plant, one may make it more or less resistant to disease as has been shown in the case of Fusarium wilt of tomato by Walker and Foster (25) and by Stoddard and Dimond (22). Resistance may also be influenced by changes in metabolism brought about by application of growth hormones. Thus, in unpublished studies made in this laboratory, a range of widely differing compounds with activity as growth hormones, which decreased the levels of reducing sugars, all increased the resistance of tomatoes to Fusarium wilt. 2,4-D was one of the compounds employed. A recent investigation by Waggoner and Dimond (24) demonstrated that maleic hydrazide increases susceptibility to Fusarium wilt. Rowell (20) has shown that 2,4-D increases susceptibility of tomatoes to early blight. The metabolic changes that increase resistance in one host pathogen combination, may increase susceptibility in another, since these relations are highly specific. The effect of 2,4-D treatment on disease is a case in point. Ibrahim (15) has recently reported a decrease in incidence of stem rust on oats following 2,4-D treatment while Hsia and Christensen (14) have clearly demonstrated an increased susceptibility of wheat to Helminthosporium. Sempio (21) has demonstrated the effects of physical and chemical factors on resistance of plants to disease and has discussed the relation of metabolism to resistance.

The host may be altered in resistance through metabolic changes brought about indirectly in the plant. Thus Keyworth and Dimond (16) have shown that chemical and physical treatments resulting in root injury to tomatoes produced a more resistant plant. Since Fusarium customarily invades the host through wounded roots, this effect appears anomalous. However, analysis of plant tissues indicated that root injury is shortly followed by marked increases in reducing sugar levels, and by decreases in amounts of certain nutrients. When plants received root injuring treatments from 10 days to 2 weeks prior to their inoculation, change in resistance was clearly evident because sufficient time had elapsed since roots were injured to shift the metabolic pattern in the plant. Conversely, when roots were injured by pruning just before inoculation, susceptibility of plants to diseases was unaltered. The root injury effect described by Keyworth and Dimond (16) was not a factor in this investigation.

Does the evidence presented here indicate that the 2 compounds 1182 and HD 160 are effective through inactivating toxins produced by Fusarium in the diseased plant? This is highly unlikely in view of the wide variety of non-fungitoxic chemical molecules which produce chemotherapeutic effects. Reactions between a chemotherapeutant and toxin must eventually rely upon chemical or specific physical reaction between compound and toxin.

The increased concentration of reducing sugar, water soluble nitrogen fractions, and rate of oxygen uptake in tomato tissue demonstrate that a metabolic change has occurred in plants treated with 1182 and HD 160. Thus, a direct correlation was observed between a change in susceptibility to disease and a modification in metabolism of the host.

SUMMARY

There is no consistent relation between fungitoxicity and chemotherapeutic activity.

Fusarium oxysporum f. *lycopersici* grew equally well on the tissue-macerate of plants treated with 1182 or HD 160 as on untreated plants. This indicates that these compounds (a) were not present in the host tissue at fungitoxic concentrations, (b) did not produce a fungitoxic component through an interaction with the host's biochemical processes and (c) did not reduce the nutritive value of the tissues to a point where it limited the growth of the parasite to a greater extent than the untreated plants.

1182 and HD 160 produced morphological and biochemical modifications in the host. 1182 increased the concentration of reducing sugar, a number of water

soluble nitrogen fractions and the rate of oxygen uptake. HD 160 increased the concentration of all the water soluble nitrogen fractions tested, but did not affect the reducing sugar content.

It is postulated that 1182 and HD 160 increased resistance to the Fusarium wilt disease of tomato by virtue of their capacity to alter the metabolism of the host.

DEPARTMENT OF PLANT PATHOLOGY
THE CONN. AGR. EXP. STATION
NEW HAVEN, CONNECTICUT

LITERATURE CITED

1. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. 1945. Official and tentative methods of analysis. Association of Official Agricultural Chemists. Ed. 6, 932 pp. Washington, D. C.
2. BARRATT, R. W. AND J. G. HORSFALL. 1947. Fungicidal action of metallic alkyl bisdithiocarbamates. Conn. Agr. Exp. Sta. Bul. 508.
3. BLANCHARD, F. A. 1951. Aureomycin chemotherapy of crown gall in tomatoes. Phytopathology **41**: 954-958.
4. BRIAN, P. W. AND JOYCE M. WRIGHT. 1951. Uptake of antibiotic metabolites of soil micro-organisms by plants. Nature **167**: 347-349.
5. BROWN, J. G. AND ALICE M. BOYLE. 1944. Penicillin treatment of crown gall. Science **100**: 528. 1944.
6. COLWELL, CHARLOTTE A. AND MARY MCCALL. 1946. The mechanism of bacterial and fungus growth inhibition of 2-methyl-1,4-naphthoquinone. Jour. Bact. **51**: 659-670.
7. CROWDY, S. H. AND R. L. WAIN. 1951. Studies on systemic fungicides. I. Fungicidal properties of the aryloxyalkylcarboxylic acids. Ann. Appl. Biol. **38**: 318-333.
8. DIMOND, A. E., D. DAVIS, R. A. CHAPMAN, AND E. M. STODDARD. Plant chemotherapy as evaluated by the Fusarium wilt assay on tomatoes. Conn. Agr. Exp. Sta. Bul. (In press)
9. FELDMAN, A. W., N. E. CAROSELLI AND F. L. HOWARD. 1950. Physiology of toxin production by Ceratostomella ulmi. Phytopathology **40**: 341-354.
10. GASSNER, G. AND W. HASSEBRAUK. 1936. Untersuchungen zur Frage der Getreideröstbekampfung mit chemischen mitteln. Phytopath. Zeit. 427-454. (Abs. 1937. In Rev. Appl. Mycol. **16**: 236.)
11. HORSFALL, J. G. AND A. E. DIMOND. 1951. Plant Chemotherapy. Trans. N. Y. Acad. Sci. **13**: 338-341.
12. HORSFALL, J. G. AND G. A. ZENTMYER. 1942. Antidoting the toxins of plant diseases. (Abs.) Phytopathology **32**: 22-23.
13. HOWARD, F. L. 1941. Antidoting toxins of Phytophthora cactorum as a means of plant disease control. Science **94**: 345.
14. HSIA, Y. AND J. J. CHRISTENSEN. 1951. Effect of 2,4-D on seedling blight of wheat caused by Helminthosporium sativum. Phytopathology **41**: 1011-1020.
15. IBRAHIM, I. A. 1951. Effect of 2,4-D on stem rust development in oats. Phytopathology **41**: 951-953.
16. KEYWORTH, W. G. AND A. E. DIMOND. 1952. Root injury as an assessment of chemotherapeutants. Phytopathology **42**: 311-316.
17. MCNEW, G. L. AND N. K. SUNDHOLM. 1949. The fungicidal activity of substituted pyrazoles and related compounds. Phytopathology **39**: 721-751.
18. MITCHELL, J. W., W. J. ZAUMEYER, AND W. P. ANDERSON. 1952. Translocation of streptomycin in bean plants and its effect on bacterial blights. Science **115**: 114-115.
19. RAWLINS, T. L. 1945. Phytopathological and botanical research methods. 156 pp. John Wiley and Sons, Inc. New York.
20. ROWELL, J. B. 1949. The pathogenicity of Alternaria Dauci f. solani. Ph.D. thesis. University of Minnesota.
21. SEMPIO, C. 1950. Metabolic resistance to plant diseases. Phytopathology **40**: 799-819.
22. STODDARD, E. M. AND A. E. DIMOND. 1948. Influence of nutritional level on susceptibility of tomatoes to Fusarium wilt. Phytopathology **38**: 670-671.
23. VICKERY, H. B., G. W. PUCHER, A. J. WAKEMAN, AND C. S. LEAVENWORTH. 1946. Chemical investigations of the metabolism of plants. I. The nitrogen nutrition of Narcissus poeticus. Conn. Agr. Exp. Sta. Bul. 496.
24. WAGGONER, P. E. AND A. E. DIMOND. 1952. Effect of stunting agents, Fusarium lycopersici and maleic hydrazide upon phosphorus distribution in tomatoes. (Abs.) Phytopathology **42**: 24.
25. WALKER, J. C. AND R. E. FOSTER. 1946. Plant nutrition in relation to disease development. III. Fusarium wilt of tomato. Amer. Jour. Bot. **33**: 259-264.
26. ZENTMYER, G. A. 1943. Mechanism of action of 8-hydroxyquinoline. (Abs.) Phytopathology **33**: 1121.

DISCOLORATION OF SOYBEAN SEEDS BY THE FROGEYE FUNGUS, CERCOSPORA SOJINA [1]

Helen S. Sherwin and K. W. Kreitlow [2]

Several organisms associated with discolorations of soybean seed in the United States have been reported. Those most commonly recognized are: *Cercospora kikuchii* (T. Matsu & Tomoyasu) Gardner, cause of purple stain; *Alternaria* sp., isolated from brown discolorations; and *Peronospora manshurica* (Naum.) Syd., causing a milky white encrustation on the seed (3). In China, Liu (8) reported approximately 12 fungi associated with discoloration of soybean seed. However, none of the discolorations previously described resembled the conspicuous discoloration observed in 2 samples of soybean seeds grown at Stoneville, Mississippi, in 1950. The frogeye leafspot fungus, *Cercospora sojina* Hara, was isolated from the affected seeds and found to be the causal agent.

Although this fungus is known to infect pods as well as leaves and stems and to be seed-borne, it has not been reported as causing a pronounced discoloration of seeds. The fungus was originally described by Hara (2) in 1915 and later by Miura (9), who named it *Cercospora daizu*. In 1928 Lehman (5) published the first account of the disease in the United States and mentioned that the fungus overwintered on seeds. In another report (6) he observed that infected seeds were sometimes noticeable because fragments of the inner lining of diseased pods frequently clung to them. In addition, there was often a slight depression on that portion of the seed which developed in contact with a lesion on the wall, and the seed surface at this point usually lacked its characteristic smoothness and luster.

DESCRIPTIONS OF DISCOLORATIONS.—The conspicuous discolorations here described were first observed in seeds of the 2 soybean selections, R46-2076 and P. I. 159,093. In R46-2076, there were 2 types of discoloration, designated as "gray" and "papillate." The discolorations in P.I. 159,093 were "brown" and "papillate." The gray discoloration varied from minute specks to graying of the entire seed coat. The color ranged from neutral to dark neutral gray sometimes with a brownish cast. The papillate spot, so called because there was usually a conical protuberance at the point of the more or less circular lesion, varied from 3 to 8 mm. in diameter. The inner 2 to 6 mm. was usually cracked, flaky, and discolored tilleul-buff to vinaceous buff. The next 0.5 to 1 mm. was colored dark vinaceous-brown while the outer edge of the lesion varied from cinnamon-brown to gray (10).

The "brown" discoloration in seeds of P.I. 159,093 varied from cinnamon-brown to Prout's brown (10), covering either a small part or all of the seed coat. Some of the lesions were zonate with alternating bands of dark and light brown. The discolored area frequently surrounded the hilum extending 4-7 mm. on either side in the form of an oval spot. Occasionally the fringe of a brown lesion diffused into a grayish color that made the gray and brown discolorations difficult to separate. The different types of discoloration are illustrated in Fig. 1.A.

Examination of several hundred diseased seed pods indicated that the papillate as well as the other discolorations developed when the fungus penetrated the pod wall usually at the suture. The papillate lesion developed where the infected lining touched a seed and, as earlier observed by Lehman, fragments of lining were sometimes found adhering to lesions on seeds.

In addition to diseased seed from Mississippi, samples of soybean seed discolored by *C. sojina* were obtained from such widely separated locations as Curtis, Louisiana; Freeburg, Illinois; Salisbury, Maryland; and Warsaw, Virginia. In these, all 3 types of discoloration occurred. Sometimes the discolorations were difficult to classify and they were then combined in a group termed "grayish-brown." The greatest amount of seed discoloration was encountered in a 1950 sample of the strain R46-2062 from Warsaw, Virginia, which contained 86 per cent diseased seeds of which 48 per cent was discolored by *C. sojina*.

These discolorations differ from those caused by *Cercospora kikuchii*, the purple stain fungus, which usually causes a pink or light to dark purple discoloration not particularly well defined as a lesion (7). In addition, *C. sojina* and *C. kikuchii* differ markedly in cultural characteristics and in spore dimensions. For comparative purposes, however, seed lots infected with each organism were used in the experiments described.

MATERIALS AND METHODS.—Several different varieties and selections of soybeans were used in this investigation. Each is listed under the appropriate experiment.

Laboratory tests were conducted to determine the extent of infection in discolored seeds and the organisms responsible. Groups of seeds representing each type of discoloration were obtained from the following seed lots: R46-2062, Warsaw, Virginia; R46-2076,

[1] Accepted for publication June 19, 1952.

[2] Assistant Pathologist and Senior Pathologist, respectively. Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Maryland.

The authors are indebted to Dr. M. G. Weiss, Soybean Project Leader, U. S. D. A., for obtaining much of the discolored seed used in this study, and to the following for sending additional samples of diseased seed: Drs. E. E. Hartwig and H. W. Johnson, Delta Agricultural Experiment Station, Stoneville, Miss.; Dr. T. J. Smith, Virginia Agricultural Experiment Station, Blacksburg, Mr. H. M. Camper, Eastern Virginia Field Station, Warsaw; Mr. J. Y. Oakes, Red River Valley Experiment Station, Shreveport, La.; and Dr. L. F. Williams, Missouri Agricultural Experiment Station, Columbia.

Stoneville, Mississippi; and P.I. 159,093, Stoneville, Mississippi. A comparable group of clean seeds was also selected from each lot. For comparative purposes purple-stained and clean seeds were selected from the lots OK-710 and L6-1152, Beltsville, Maryland. The severely diseased lot R46-2062 contained 28 per cent papillate, 20 per cent brown, and approximately 38 per cent miscellaneous discolored seeds including purple. R46-2076 contained 33 per cent discolored seeds of which 15 per cent were gray, 6 per cent papillate and the remainder miscellaneous including purple. P.I. 159,093 contained 14 per cent discolored seeds of which 6 per cent were brown and the remainder miscellaneous including purple. OK-710 had 8 per cent purple seeds and 2 per cent miscellaneous discolorations. L6-1152 contained 19 per cent discolored seeds of which 18 per cent was purple and the remainder miscellaneous discolorations.

The different samples of discolored and clean seeds were surface sterilized for 1, 5, 10, 15, and 20 minutes in 1:500 mercuric chloride in 50 per cent ethyl alcohol. Following surface sterilization the seeds were rinsed in several changes of sterile water and then plated aseptically in Petri dishes of Difco potato-dextrose agar that contained 50 mg./liter of the ammonium salt of 2,4-D to inhibit germination of the seeds. This medium was a modification of that described by Hagborg et al (1).

The relationship of discoloration to emergence of seedlings was studied in greenhouse and field experiments. For the greenhouse tests, 50 seeds of each type of discoloration were selected from lots R46-2076, gray and papillate; P.I. 159,093, brown; and OK-710, purple. A comparable number of clean seeds was selected from each sample.

Each group of seeds was then planted in 4-in. pots of steamed soil, 5 seeds to a pot. Germination, development of abnormal growth, and presence of lesions on cotyledons or other parts of seedlings was recorded.

For the field test, 500 papillate and grayish-brown discolored seeds were selected from each of 2 lots of R46-2062. One lot was obtained from Stoneville, Mississippi, and the other from Warsaw, Virginia. Similarly 500 purple-stained seeds were selected from each of 2 lots of the varieties Lincoln and Wabash, grown at Beltsville, Maryland. For comparison, 500 clean seeds were selected from each of the 4 samples. Each group of 500 seeds was planted in 4 10-ft. rows (125 seeds per row). The rows were distributed at random in the plot. Seeds were planted May 21, 1951, and stand counts recorded June 12.

Artificial inoculation with cultures obtained from discolored seeds was conducted in both greenhouse and field. For some of the tests, the fungus was grown 2 weeks in 250 ml. Erlenmyer flasks containing 30 ml. of carrot decoction.[3] The mycelial mat that developed was macerated in a Waring blendor and the suspension was atomized on test plants. For 1 of the field tests, *C. sojina* was grown on a mixture of wheat and oats. The grain cultures were dried, pulverized, and screened through a 100-mesh sieve. The ground inoculum was dusted on leaves moistened with water from a sprayer (4).

TABLE 1.—*Percentage of discolored and clean soybean seeds infected with Cercospora sojina and Cercospora kikuchii following surface sterilization* [a]

Treatment time in minutes	Cercospora sojina				Cercospora kikuchii	
	Gray	Brown	Papillate	Clean	Purple	Clean
1	85	80	85	13	85	0
5	50	65	70	.05	69	0
10	45	60	77	.03	54	.04
15	—	53	60	.03	48	0
20	—	36	—	.06	30	0

[a] Percentages based on 20–80 seeds for each treatment.

Seedlings inoculated in the greenhouse were usually incubated at 25-27° C. in a moist chamber for 2-3 days. Pods on older plants were inoculated by rubbing with a cheesecloth pad soaked in the mycelial suspension. Pods on check plants were not inoculated. The older plants were incubated for several weeks in a room at 25-27° C. maintained at high humidity and artificially lighted by 2 banks of 44 standard, cool white, 96-inch, T8 fluorescent lamps.

Field inoculations were made on plants of the susceptible varieties Gibson, Hawkeye and Patoka. One-half of the plants were sprayed with a suspension of the fungus and the remainder were inoculated by dusting them with pulverized grain inoculum.

RESULTS.—*C. sojina* was recovered from a high percentage of discolored seeds surface sterilized for 1, 5, 10, 15, and 20 minutes, but from a very low percentage of clean seeds similarly surface sterilized (Table 1). This suggests that in the discolored seeds mycelium of the fungus frequently extends beneath the seed coat to the cotyledons and perhaps the embryo, while infection in clean seeds is usually superficial if present at all. Surface sterilization of purple-stained seeds for 20 minutes failed to eliminate all the fungus.

In the greenhouse tests fewer seedlings developed from the gray, papillate and brown discolored seeds than from clean seeds (Fig. 1,B). Seedlings from diseased seeds had more cotyledonary lesions and abnormal growth than those developing from clean seeds (Table 2). In contrast, there was little difference in seedling emergence from purple-stained and clean seeds; however, more seedlings with cotyledonary lesions and abnormal growth developed from the purple discolored than from the clean seeds.

In field tests, seedling emergence from discolored and clean seeds substantiated results obtained in greenhouse experiments (Table 3). Seedling emergence from both samples of papillate and grayish-brown dis-

[3] The decoction was prepared by autoclaving 250 g. of chopped carrots in 1000 ml. of distilled water for 15 minutes at 15 lbs. pressure. The liquid portion was decanted and 10 gm. of dextrose added before the medium was dispensed into flasks and sterilized.

A

B

C

TABLE 2.—*Percentage of seedling emergence from discolored and clean soybean seeds planted in the greenhouse*

	R46-2076				P.I. 159,093		OK-710	
	Gray		Papillate		Brown		Purple	
	Discolored	Clean	Discolored	Clean	Discolored	Clean	Discolored	Clean
Per cent emerged	82	96	90	98	54	86	96	96
Per cent dwarfed or with abnormal growth	48	2	16	8	34	6	18	2
Per cent with cotyledonary lesions	78	24	90	22	54	16	66	6

colored seeds was significantly less than from comparable samples of clean seeds. The results of seedling emergence from purple-stained and clean seeds were variable. In 1 sample seedling emergence from purple-stained seeds was slightly less, but not significantly so, and in the other greater than the check.

Conspicuous frogeye symptoms developed on leaves of seedling plants 2 weeks following artificial inoculation in the greenhouse. Lesions on pods required 17 to 21 days to develop (Fig. 1,C). Seeds in immature pods were rarely discolored and then only if situated beneath a severe lesion. As diseased pods matured and the fungus penetrated further, typical gray, brown, and papillate discolorations developed on the seeds. The amount of discoloration produced by artificially inoculating pods with *C. sojina* varied from 20 per cent in a susceptible variety to none in a resistant one. Seeds from non-inoculated pods even in susceptible varieties were free from discoloration caused by *C. sojina*.

Lesions did not develop on pods of plants inoculated in the field until the plants approached maturity. In general, no seed discolorations were found directly beneath young lesions. As the pods matured, the lesions enlarged and seed discolorations were found more frequently.

Following maturity, plants were harvested 3 times at 2-week intervals to see if there was an increase in amount of discolored seeds when plants were left standing in the field. There was 20 per cent discolored seeds in the variety Hawkeye on September 19, 44 per cent 2 weeks later, and 37 per cent in the third harvest. For the variety Patoka there was 10 per cent discolored seeds October 1, 48 per cent 2 weeks later, and 28 per cent at the last harvest. The variety Gibson had 10 per cent discolored seeds October 1, and 18 and 27 per cent in the second and third harvests respectively. The percentages were based on 1,000 seeds examined from each lot. There was an increase in per cent discolored seeds in the second harvest over the first in all 3 varieties, but only the variety Gibson yielded more discolored seeds in the third harvest than in the second. One explanation of this might be that some of the diseased pods shriveled and dropped or scattered discolored seeds before the third harvest. Only 1.58 inches of rain fell during the 6-week period between the first and last harvest. If more rain had fallen there might have been a greater increase in seed discoloration between the first and last harvests.

DISCUSSION.—Seed infected with *Cercospora sojina* is probably responsible for initiating many field infections of the frogeye disease. The fungus was found sporulating abundantly in the greenhouse and field in lesions on cotyledons of seedlings that developed from discolored seeds. Usually the fungus fruited on fragments of the diseased seed coat that adhered to the cotyledons. The spores produced presumably infected the foliage since it was observed in the field that le-

TABLE 3.—*Percentage of seedling emergence from discolored and clean soybean seeds planted in the field*[a]

Variety or numbered selection	Type of discoloration	Source	Per cent germination
R46-2062	Papillate and grayish-brown	Stoneville, Miss.	41.20 }**
R46-2062	Clean	Stoneville, Miss.	68.80 }
R46-2062	Papillate and grayish-brown	Warsaw, Va.	41.40 }**
R46-2062	Clean	Warsaw, Va.	77.20 }
Lincoln	Purple	Beltsville, Md.	83.00
Lincoln	Clean	Beltsville, Md.	86.00
Wabash	Purple	Beltsville, Md.	91.60 }*
Wabash	Clean	Beltsville, Md.	83.60 }

[a] Four replications of 125 seeds each were planted for each seed lot on May 21, 1951, and emergence counts were made June 12, 1951.

* Significant at the 5 per cent level.

** Significant at the 1 per cent level.

FIG. 1. A, Seeds of soybean discolored by *C. sojina*. (Left to right) gray, brown, papillate, and clean; B, Emergence of brown discolored (left) and clean (right) seeds of soybeans planted in pots of steamed soil; C, Discoloration of soybean pod and a seed artificially inoculated with *C. sojina*. A healthy pod and seeds are on the right.

sions appeared first on leaves of plants from diseased seeds and later on foliage of plants from healthy seeds that were growing in adjacent rows.

Lehman was unable to find lesions unquestionably those of the frogeye fungus on cotyledons of plants in the field (6). The seed with which he worked was not conspicuously discolored and apparently was not so severely diseased as that encountered in studies reported here. By germinating diseased seeds in large test tubes Lehman found the fungus sporulated on the seed coat which clung to the cotyledons and he concluded that the spores are transported thence to the true leaves by wind and other agencies. It is evident from the results of tests described in this study that the fungus is deep-seated in some of the seeds. Lehman (6) found that the fungus in diseased seeds occasionally survived as much as 60 minutes surface sterilization in 1:1000 mercuric chloride but in a large percentage of seeds was so superficial as to be killed by seed disinfection. Our tests showed that in a high percentage of discolored seeds, which were severely infected, the fungus would survive 20 minutes surface sterilization in 1:500 mercuric chloride in 50 per cent ethyl alcohol. These results indicate that seed treatment might not be effective in eliminating the organism from all discolored seeds.

SUMMARY

Conspicuous discolorations designated as "gray," "brown," or "papillate" were found in seed of soybeans grown in Illinois, Louisiana, Maryland, Mississippi, and Virginia. The discolorations were caused by the frogeye fungus *Cercospora sojina* and differed markedly from the purple stain discoloration caused by *C. kikuchii*. Surface sterilization of diseased seeds for 20 min. in 1:500 mercuric chloride in 50 per cent ethyl alcohol failed to eliminate all the fungus.

In greenhouse and field tests seeds discolored by *C. sojina* failed to germinate as well as clean seeds. In addition, many of the seedlings that originated from discolored seeds bore lesions on cotyledons and developed earlier infection on leaves in the field. In contrast, seeds infected with the purple stain fungus germinated normally and produced stands comparable to those from clean seeds. The seed discolorations were reproduced artificially by inoculating pods of plants growing in the greenhouse and in the field with cultures of *C. sojina* isolated from discolored seeds. In the field there was a tendency toward progressive increase in seed discoloration when plants were left standing after they matured.

PLANT INDUSTRY STATION,
BELTSVILLE, MD.

LITERATURE CITED

1. HAGBORG, W. A. F., GLADYS M. WARNER, AND N. AGNES PHILLIPS. 1950. Use of 2,4-D as an inhibitor of germination in routine examinations of beans for seed-borne infection. Science **111**: 91.
2. HARA, K., 1915. (Spot disease of soybean.) Agr. Country **9**: 28. (In Japanese).
3. JOHNSON, H. W. AND B. KOEHLER. 1943. Soybean diseases and their control. U. S. Dept. Agr. Farmers' Bul. 1937.
4. KREITLOW, K. W. 1951. Infection studies with dried grain inoculum of Sclerotinia trifoliorum. Phytopathology **41**: 553-558.
5. LEHMAN, S. G. 1928. Frogeye leaf spot of soybean caused by Cercospora daizu Miura. Jour. Agr. Res. [U.S.] **36**: 811-833.
6. LEHMAN, S. G. 1934. Frogeye (Cercospora daizu Miura) on stems, pods, and seeds of soybean, and the relation of these infections to recurrence of the disease. Jour. Agr. Res. [U.S.] **48**: 131-147.
7. LEHMAN, S. G. 1950. Purple stain of soybean seeds. North Carolina Agr. Exp. Sta. Bul. 369.
8. LIU, S. T., 1948. Seed-borne diseases of soybean. Bot. Bul. Acad. Sinica **2**: 69-80.
9. MIURA, M., 1921. Diseases of the main agricultural crops of Manchuria. So. Manchuria Railway Co. Agr. Exp. Sta. Bul. 11, 56 p. Illustrated. (In Japanese. English abstract in Japan. Jour. Bot. **1** (1): (9), 1922.)
10. RIDGWAY, R., 1912. Color standards and color nomenclature. Washington.

COMMON LETTUCE MOSAIC AND ITS CONTROL BY THE USE OF MOSAIC-FREE SEED [1]

R. G. Grogan, J. E. Welch and Roy Bardin

Common mosaic of lettuce was first recognized and described by Jagger in Florida in 1921 (7) and shown to be seed transmitted by Newhall in 1923 (9). This virus disease has been considered of minor importance in the United States. Broadbent (3) reported that losses of ¼ of the crop are common in southern England and occasionally an entire crop is rendered unmarketable. He stated that the disease has been recognized in Britain since 1931 but is widely and erroneously considered due to adverse weather, unfavorable soil conditions, or insect damage. It seems probable that a similar assumption, at least in part, has prevailed in the United States since in some years the spring and late fall lettuce crops in California have been damaged severely by comon lettuce mosaic. According to Thompson[2] the disease has caused considerable damage to the lettuce crop in some eastern states.

Newhall (9) showed that a relatively small percentage of seed-borne virus was the most important source of primary inoculum, and suggested that the disease might be controlled by use of virus-free seed. The present paper describes a method of producing mosaic-free seed and presents data on the efficacy of clean seed in controlling the disease.

SYMPTOMATOLOGY.—For the most part, the described symptoms of lettuce mosaic have been those occurring on varieties other than the Imperial or Great Lakes crisphead types which are used for the bulk of the lettuce acreage of the United States. Symptoms of the disease are quite different in young and in mature plants and also vary somewhat in different varieties. It seems desirable, therefore, to describe the symptoms of common lettuce mosaic of the crisphead varieties in all stages of growth, ranging from small seedlings to seed-bearing plants.

Symptoms in young plants—The first symptom of mosaic in seedling plants is a slight inward rolling of the leaves along the long axis, and the first true-leaf is often irregularly shaped and slightly lobed. These symptoms are followed or accompanied by a light green to yellow mottling (Fig. 1A). Later, in the young rosette stage, the mottled leaves may show vein clearing and slight bronzing (Fig. 1C)[3]. The latter is due to necrotic flecking of the leaf blade especially along the smaller veinlets; the intensity of the symptom varies with the variety and with certain undefined climatic factors.

Symptoms in older plants—In older plants the mottle symptom which was evident in seedling and young plants is usually absent or indistinct and other symptoms must be relied upon for recognition of the disease. The disease in half-grown to mature plants, i.e., ones approaching marketable age, is evidenced by a severe stunting and a dull-green to slightly yellow discoloration of the whole plant. Tips of the outer leaves are rolled downward giving the plants a wilted appearance although the leaves may be quite turgid. Mottling, if still distinguishable, may be seen best near the margins of the leaves (Fig. 1 E and F).

Most mosaic-infected plants, especially those which were infected early, are extremely stunted and fail to head normally. The midribs of these stunted plants protrude abnormally on the underside of the leaf blades giving the underneath portion of the heads a ribby appearance. This symptom is especially prominent in the Great Lakes variety.

Symptoms in seed plants—In seed fields mosaic is easily recognized by a distinct mottling of the younger leaves of diseased plants which have produced seed-stalks. Because this condition is so common in commercial seed fields, it is regarded by some as the normal appearance of seed lettuce plants.

Symptom variation of varieties and individuals—The symptoms described above are typical of lettuce mosaic in most crisphead varieties. However, certain varieties, such as Imperial D and Eiffel Tower Cos, are hypersensitive to the virus and when infected develop severe necrosis of the leaf blade (Fig. 1D). Occasional plants of the Great Lakes and Imperial 615 varieties, in which the typical varietal symptom of lettuce mosaic is mottling, stunting, and rolling of leaves, may show necrosis similar to the typical necrotic symptom of Imperial D and Eiffel Tower. Such necrotic symptoms are often difficult to distinguish from early symptoms of spotted wilt.

EPIDEMIOLOGY—Several workers (1, 3, 5, 8) have confirmed Newhall's report (9) that common lettuce mosaic is seed transmitted. While seed transmission occurs to the extent of about 3 per cent, considerable variation exists among varieties with respect to this characteristic. For example, the variety Bibb may have 8 per cent or more infected seed while the variety Cheshunt Early Giant was reported by Kassanis (8) to be entirely free of seed-borne virus. Tests of standard varieties used in California have shown that the percentage of infected seed usually varies from 1 to 3 per cent. No commercial seed samples tested have

[1] Accepted for publication June 30, 1952.

The writers are indebted to W. C. Snyder for helpful suggestions and encouragement during the course of this work and to J. W. Kever for assistance in maintaining the experimental plots.

[2] Information obtained by conversation with Ross C. Thompson, Senior Horticulturist, Division of Vegetable Crops and Diseases, USDA, Beltsville, Maryland.

[3] The big-vein disease of lettuce occasionally has been confused with mosaic. However, the vein clearing typical of big vein is much more pronounced than that of mosaic and is the principal symptom of the disease. Under California conditions, especially in the spring crop, it is common for both diseases to occur in the same plant.

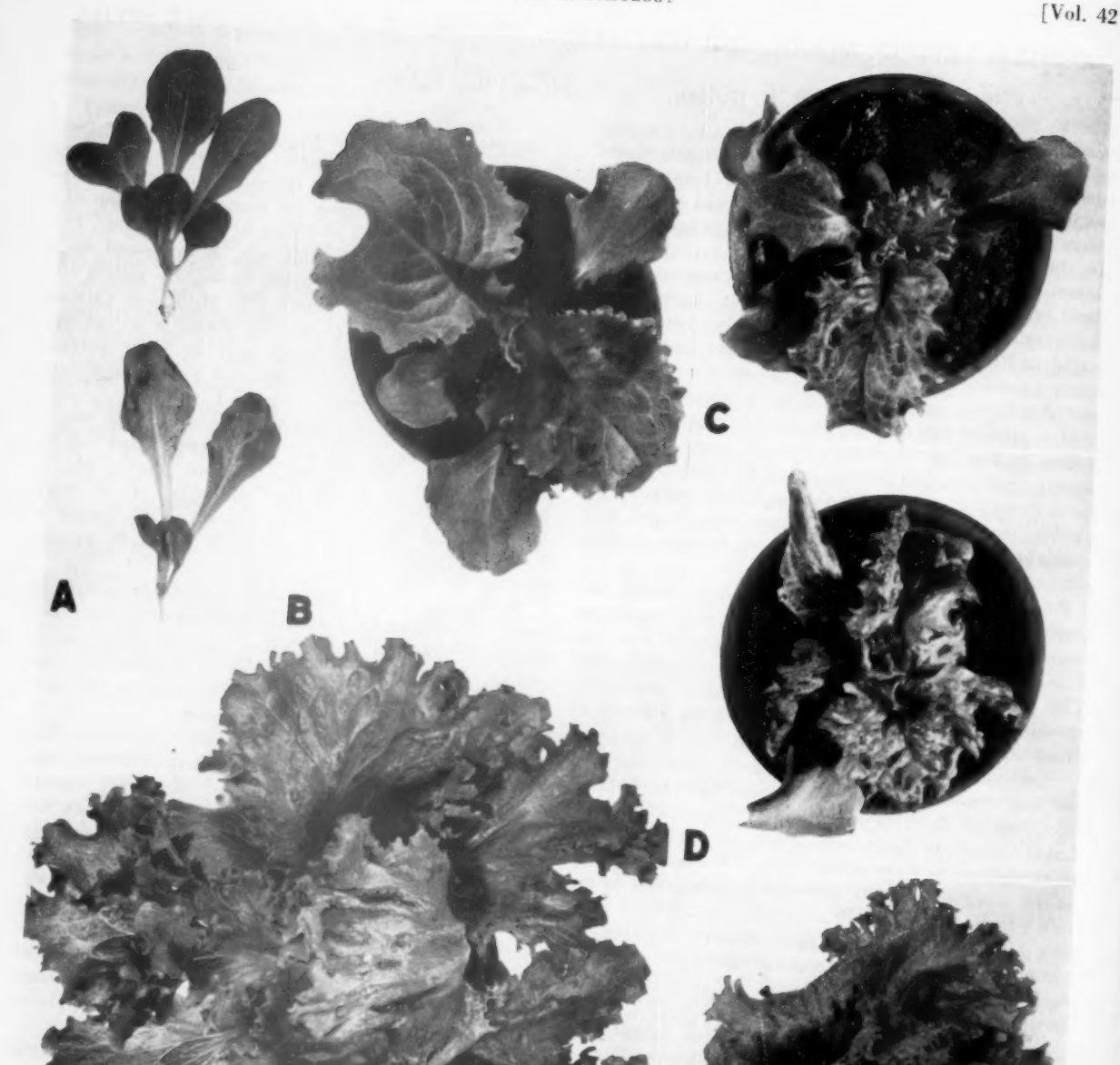


FIGURE 1. Symptoms of common lettuce mosaic in plants ranging in age from seedlings to nearly mature heads. A) Upper seedling healthy—lower seedling diseased as a result of seed transmission. Note irregular shape, rolling and mottling of leaves. B, C, and D) Young rosette stage—B) healthy. C) Plant showing mottling and vein clearing. D) Plant showing necrosis of leaf blade. E) Healthy plant approaching head maturity. F) Mosaic-infected plant of same ages as E. Note dull green color, stunting, and downward rolling of leaves.

been entirely free of the virus. Tests of seed samples from single plants known to be infected yielded approximately the same percentage of seed-borne infected seedlings as commercial seed samples, indicating that commercial seed is usually obtained from fields which were almost 100 per cent infected. This

hypothesis has been checked and confirmed on several occasions by examination of commercial seed fields.

Use of mosaic-infected seed results in an abundant source of primary inoculum scattered uniformly over the field. By actual count it has been found that, at average seeding rates, there is 1 seed-borne mosaic infected plant in every 4 ft. of bed[4] before thinning. However, secondary spread by aphids from the seed-borne infected plants to adjacent healthy plants must take place before serious economic damage occurs. In the Imperial Valley of California the crop is planted in the fall following extremely high summer temperatures which have reduced the aphid population practically to zero. The crop grows and reaches head maturity during the winter months when temperatures are too low for aphid build-up and movement. The disease is therefore of little economic importance in the Imperial Valley because, for the most part, the only plants infected with mosaic are those originating from infected seed. However, if these same fields are allowed to produce seed, the growing period extends into the late spring when aphid build-up and movement is at its peak. Under these conditions practically all the plants contract mosaic and the resulting seed has the usual percentage of seed-borne mosaic.

In contrast to the Imperial Valley, the Salinas Valley and other cool coastal areas of California produce lettuce crops for spring, summer and fall harvest. The spring crop is often affected severely by mosaic because most of the growth occurs during the spring, at which time aphid build-up and movement is at its peak. Consequently, the initial seed-borne mosaic is spread to the surrounding plants and infection often reaches 30 per cent or more soon after thinning; at time of head maturity almost all plants in such fields may be infected. Under these circumstances the disease causes a serious reduction in yield of marketable heads.

Aphid populations in this same area during late June, July and August are usually very low and mosaic causes little appreciable damage to the crop grown for summer harvest. During September and October, however, there is usually another build-up of aphids, and mosaic oftentimes causes serious losses in the crop grown for fall harvest.

Broadbent (3) described a somewhat similar pattern for the build-up and decline of aphid populations in southeast England which also determined the severity of lettuce mosaic. He postulated that predators and parasites and possibly the heat from the soil surface in sunny weather might explain the reduced aphid populations during the summer. These same factors probably govern to a large extent the prevalence of aphids at different times of the year in the coastal areas of California.

[4] The usual lettuce bed is a raised ridge 20 in. across the top on which 2 rows are planted 14 in. apart. Plants are thinned to intervals of about 1 ft. The distance from center to center of adjacent beds including the irrigation furrow is 40 in.

PRODUCTION OF MOSAIC-FREE SEED.—It has been suggested (1, 4, 8) that lettuce mosaic could be controlled by the use of virus-free seed. However, insofar as the writers are aware no one has actually tested experimentally this obvious control measure.

Mosaic-free seed stock was produced by the following method: commercial seed of the varieties Great Lakes and Imperial 615 were seeded in flats in an aphid-free greenhouse. After the seedlings had reached the first true-leaf stage they were transplanted into small paper cups. During a 3-weeks period thereafter the plants were inspected carefully on 3 separate occasions and all plants that showed symptoms of mosaic were removed and destroyed. The remaining plants were then transplanted to fields which were isolated from the nearest commercial lettuce growing area by about 15 miles. After transplanting no attempt was made to control aphids by spraying or dusting, but no plants developed mosaic during the growing season. Seed was harvested and tested in the greenhouse for seed-borne mosaic and none was found.

The most important factor in the selection of an area for the production of mosaic-free seed is sufficient isolation from commercial lettuce producing areas. However, since lettuce mosaic virus has several hosts other than cultivated lettuce, the presence and prevalence of these other hosts is important. Ainsworth and Ogilvie (1) reported that *Senecio vulgaris* L., *Sonchus asper* L., *Lathyrus odoratus* L., and *Pisum sativum* L. var. Lincoln were susceptible while *Sonchus oleraceus* L., *Sonchus arvensis* L., *Taraxacum officinale* Weber and *Carduus arvensis* Robson were not. Kassanis (8) reported that *Lactuca serriola* L., prickly lettuce, and *Lactuca virosa* L. are susceptible. Endive, Aster, Cineraria and Marigold have also been reported susceptible to infection by artificial inoculation (2). No extensive study of the host range of lettuce mosaic virus has been made by the present writers. However, 2 previously unreported hosts, zinnia, *Zinnia elegans* Jacq., and Witloof chicory, *Cichorium intybus* L., were found to be susceptible when inoculated mechanically. Several California collections of *Sonchus* spp. have been tested by mechanical inoculation but no infection resulted. Field observations also indicated that *Sonchus* spp. common to California are not susceptible to mosaic. Prickly lettuce, *L. serriola*, is by far the most prevalent and widely distributed wild host of lettuce mosaic in California. However, unless growing near plantings of cultivated lettuce, it has been found without exception to be free of mosaic. On the other hand, when growing near cultivated lettuce it usually showed mosaic infection. Seed harvested from mechanically infected prickly lettuce plants failed to show any seed transmission in more than 900 seedlings. This indicates that the virus is rarely, if ever, transmitted in prickly lettuce seed, which probably explains why the virus has not been found in this species except when growing near cultivated lettuce.

Prickly lettuce is, however, frost hardy and plants

which grow from seed germinated by fall rains may remain alive throughout the winter. This overlapping of generations may perpetuate lettuce mosaic for a number of years after commercial lettuce production in the area. While this fact presents a danger in selecting suitable areas for the production of virus-free seed, it also affords a reliable test for determining a virus-free area in the absence of previous crop history. If the wild prickly lettuce shows mosaic infection, the production of virus-free seed in the area would be unsuccessful except in the absence of aphids.

TEST OF MOSAIC-FREE SEED IN COMMERCIAL PLANTINGS.—The efficacy of clean seed as a control of lettuce mosaic was tested by planting several plots in commercial fields in the Salinas Valley for the 1951 spring harvest. Except where otherwise noted these plots were approximately 180 ft. square and were usually situated near the centers of large commercial fields planted at the same time and with the same lettuce variety as the mosaic-free seed. Care was taken to avoid contamination of the plot area with commercial seed by covering the shoes of the cooperator's planter with small cloth sacks and by cleaning off all loose seed from the planting sled each time before it ran through the area reserved for the plot. The plot was then planted with mosaic-free seed.

Counts of mosaic infected and total number of plants were made in 180 ft. of nine beds on each side of the plot and in all beds inside the plot. This afforded a comparison between 2 9-bed groups on each side of the plot planted with commercial seed and the 9-bed groups within each plot planted with mosaic-free seed.

The first plot was planted December 13, 1950, near Gonzales, California, and the data obtained are in Table 1.

Data from plot 1 show a considerably lower percentage of mosaic-infected plants in the plot planted with clean seed than in the 9 beds on each side planted with commercial seed. In the early stages of growth, no mosaic plants were found within the plot, whereas approximately 2 per cent of the plants in the commercial field were infected. This initial infection is believed to have originated from infected seed. By March 24 there was a slight increase in the percentage of mosaic in the commercial planting and a small amount had spread into the plot. At the time of the final reading on April 17, just prior to harvest, the percentage of infection within the plot was still very low, about 2 per cent, but infection in the commercial field had increased to about 12 per cent. There was a striking difference in the percentage of mosaic-infected plants in the 2 adjacent individual beds inside and outside the plot. The first bed on the west side of the commercial field showed 14.9 per cent mosaic plants whereas the first bed inside the plot showed only 2 per cent mosaic plants. This indicated that aphid movement was for the most part from plant to plant down the bed rather than across the furrow.

TABLE 1.—*The incidence of mosaic infection in plot 1 planted with mosaic-free seed as compared with that in 9 commercial lettuce beds on each side* [a]

Location of beds	Number of mosaic plants in approximately 2700		Per cent mosaic infection	
	3/24	4/17	3/24	4/17
1st 9 beds west and outside of plot	69	302	3.1	11.2
1st 9 beds in plot	0	50	0.0	1.8
2nd 9 beds in plot	4	59	0.1	2.1
3rd 9 beds in plot	2	48	0.05	1.7
4th 9 beds in plot	3	58	0.1	2.1
5th 9 beds in plot	5	58	0.2	2.1
6th 9 beds in plot	3	61	0.1	2.2
1st 9 beds east and outside of plot	57	355	2.1	13.2

[a] The direction of the prevailing wind was parallel to the direction of the beds.

TABLE 2.—*The incidence of mosaic infection in plot 2 planted with mosaic-free seed as compared with that in 9 commercial lettuce beds on each side* [a]

Location of beds	Number of mosaic plants in approximately 2000		Per cent mosaic infection	
	4/4	4/24	4/4	4/24
1st 9 beds windward (north) and outside of plot	313	722	12.7	30.2
1st 9 beds in plot	53	133	2.5	6.5
2nd 9 beds in plot	21	72	1.0	3.7
3rd 9 beds in plot	12	54	0.5	2.7
4th 9 beds in plot	8	22	0.4	1.2
5th 9 beds in plot	14	33	0.7	1.8
1st 9 beds leeward (south) and outside of plot	174	262	7.3	11.1

[a] Direction of prevailing wind was at right angles to direction of the beds.

Data from plot 2, which was planted December 18, 1950, near Salinas, California, are in Table 2.

The data in Table 2, like those of Table 1, show that a much higher percentage of mosaic infection occurred in the commercial field than in the virus-free-seed plot. On April 24, just prior to harvest, approximately 30 per cent of the plants in the commercial beds bordering the plot on the windward side were infected with mosaic, while only 11 per cent of the plants in the beds bordering the leeward side were infected. This same relationship was also apparent within the plot inasmuch as there was a gradual reduction from 6.5 per cent infection in the first 9-bed group on the windward side to only 1.8 per cent infection in the corresponding group on the leeward side, the average being 3.2 per cent. The high percentage of mosaic on the windward side resulted from a heavy migration of aphids from a nearby field of weeds which was disced when the lettuce was in the early rosette stage.

TABLE 3.—*A distribution map showing percentage of mosaic infected plants in consecutive 100-plant groups in plot 3 planted with mosaic-free seed as compared with similar counts in 3 adjacent beds planted with commercial seed* [a]

Position of plant groups relative to old lettuce field on windward side of the plot	Commercial seed			Mosaic-free seed					
	Bed			Bed					
	3	2	1	1	3	5	10	11	12
1st	93	92	98	91	92	83	85	80	99
2nd	91	89	94	84	89	73	72	78	81
3rd	89	89	86	86	79	72	48	59	74
4th	95	92	91	79	78	51	39	53	54
5th	92	92	97	76	78	55	44	17	66
6th	91	88	90	62	70	56	21	17	24
7th	92	90	83	53	83	45	25	10	18
8th	88	83	85	51	58	72	11	18	20
9th	87	76	84	50	61	38	8	6	30
10th	89	79	81	46	30	62	10	8	19

[a] The plant groups are numbered from the windward side adjacent to an old lettuce field. The beds in which the mosaic percentage counts were made were approximately 700 ft. long and parallel to the direction of the prevailing wind.

Plot 3 was planted March 22, 1951, near Watsonville, California, and the data are in Table 3.

Unlike plots 1 and 2, plot 3 was a 6-acre triangular section of a 14-acre field and was bordered on the windward side by an old lettuce field. This field, which was on a level approximately 20 ft. below the test plot, was disced 9 days after the test plot was thinned.

It is evident from the data in Table 3 that a considerable number of viruliferous aphids migrated into the test field from the old lettuce planting on the windward side. However, considering the field as a whole, there was a noticeable decrease in the percentage of mosaic from the windward to the leeward side of the test plot which was not reflected in the commercial planting. For example, bed 3 inside the plot had 92 per cent infected plants in the first group while there were only 30 per cent infected plants in the 10th group. In contrast, bed 3 in the commercial field had 93 per cent infected plants in group 1 and 89 per cent in the 10th group. This decrease in percentage of mosaic infection from windward to leeward was even more pronounced in beds 10, 11 and 12 which were separated from the adjacent commercial planting by several beds planted with mosaic-free seed. This was probably due to a partial elimination of the border effect from the commercial field.

Plot 3 represents as severe a test of the efficacy of mosaic-free seed for the control of lettuce mosaic as is likely to be encountered under natural field conditions. Although the area planted with virus-free seed showed a relatively high percentage of mosaic due to the migration of viruliferous aphids from the adjacent older lettuce planting, the 6-acre test plot yielded 238 crates per acre as against 100 crates per acre in the commercial field. Had the test plot been small the benefit of clean seed might not have been evident.

DISCUSSION.—The results of this investigation on the origin and spread of lettuce mosaic in the field agree, for the most part, with those of Broadbent et al (4). While these workers recognized that infected seed was the most important source of primary inoculum, they concluded that spread of the virus from older plantings to younger fields was of more importance than was found in the present study. They based their conclusions on the fact that the edges of fields were more heavily infected with mosaic than were the interior portions of the same fields. Similar observations were made in the present study, but since areas planted with mosaic-free seed showed a much lower percentage of mosaic than the adjacent areas planted with commercial seed, it was concluded that the higher percentage of mosaic at the border did not necessarily mean that all the aphids were viruliferous at the time of migration, but simply that the concentration of aphids was greater in that part of the field. Since an abundant source of primary inoculum is always present throughout a planting made with infected seed, a heavy aphid infestation at any place in the field will increase the incidence of the disease. This point would be overlooked unless a planting of mosaic-free seed was available for comparison.

The results of the present work tend to confirm the conclusion reached by Kassanis (8) that spread of common lettuce mosaic is mainly a local phenomenon and is therefore amenable to control by the use of mosaic-free seed.

SUMMARY

The symptoms of common lettuce mosaic as it affects the crisphead varieties in all stages of growth are described and the epidemiology of the disease under California conditions is discussed. It has been known for many years that common lettuce mosaic virus is seed-borne and several workers have suggested the use of disease-free seed as a control. However, this method of control had not been tested experimentally. A sample of mosaic-free seed was produced by roguing all seed-borne infected plants under aphid-free greenhouse conditions after which the remaining healthy plants were grown to maturity in an isolated area. The resulting seed proved free of mosaic and was used to plant experimental plots in commercial fields. Results of field trials showed that the spread of common mosaic under California conditions is mainly a local phenomenon and that the use of mosaic-free seed is an effective means of controlling the disease in large scale field plantings.

DIVISION OF PLANT PATHOLOGY
AND
DIVISION OF TRUCK CROPS
UNIVERSITY OF CALIFORNIA, COLLEGE OF AGRICULTURE, DAVIS, CALIFORNIA
AND
MONTEREY COUNTY, DEPARTMENT OF AGRICULTURE
SALINAS, CALIFORNIA

LITERATURE CITED

1. Ainsworth, G. C., and Ogilvie, L. 1939. Lettuce mosaic. Ann. Appl. Biol. **26**: 279-297.
2. Anonymous. 1949. Twenty-third annual report of the department of Scientific and Industrial Research, New Zealand. 88 pp. (R.A.M. **30**: 19-20. 1951).
3. Broadbent, L. 1951. Lettuce mosaic in the field. Agriculture: Jour. Ministry Agri. **57**: 578-582.
4. Broadbent, L., T. W. Tinsley, W. Buddin and E. T. Roberts. 1951. The spread of lettuce mosaic in the field. Ann. Appl. Biol. **38**: 689-706.
5. Grogan, R. G., and Roy Bardin. 1950. Some aspects concerning seed transmission of lettuce mosaic virus (Abs.) Phytopathology **40**: 965.
6. Grogan, R. G., J. E. Welch and Roy Bardin. 1951. The use of mosaic-free seed in controlling lettuce mosaic (Abs.) Phytopathology **41**: 939.
7. Jagger, I. C. 1921. A transmissible mosaic disease of lettuce. Jour. Agr. Res. (U.S.) **20**: 737-741.
8. Kassanis, B. 1947. Studies on dandelion yellow mosaic and other virus diseases of lettuce. Ann. Appl. Biol. **34**: 412-421.
9. Newhall, A. G. 1923. Seed transmission of lettuce mosaic. Phytopathology **13**: 104-106.

A PHYTOPATHOLOGICAL NOTE

Sori of Gladiolus Smut in the White Flesh of Gladiolus Corms. Donald P. Limber. In an earlier volume of Phytopathology the writer illustrated and described the sori of gladiolus smut, *Urocystis gladiolicola* Ainsworth, on gladiolus corm scales.[1] Since that time other shipments of smutted gladiolus which have carried the infection in the flesh of the corms have been received at the Bureau of Entomology and Plant Quarantine inspection house at Hoboken, New Jersey. The destructive nature of the disease becomes apparent when such corms are examined, as the corm may be reduced to a mummy enclosing a mass of smut spore balls.

MacLean[2] has recently reported the presence of gladiolus smut in the San Francisco Bay area. It therefore becomes important that gladiolus growers and plant pathologists have reference illustrations of the disease as it appears in the corms to supplement those showing the symptoms on the corm scales.

Figure 1 shows a series of corms in various stages of the disease. Small lesions are covered by unbroken epidermis and show on the surface as bluish-black spots. The larger lesions nearly always arise in the basal plate and extend upward through the core, as shown in Figure 1, B and C. The decay then spreads into the surrounding tissue, and at the last the whole corm may be affected, as in Figure 1 A.

Smut should not be confused with *Papulaspora* spp., which have similar spore balls. The smut sori on the corm scales are distinctive. *Papulaspora* does not produce a true sorus. In the flesh of the corm, *Papulaspora* is associated with brown rather than black decay, and its spore balls can be seen, with a hand lens, to be borne on loose mats of brown mycelium. Gladiolus smut produces dense, black masses of spore balls in which the mycelium is little in evidence.—Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, U. S. Department of Agriculture, Hoboken, New Jersey.

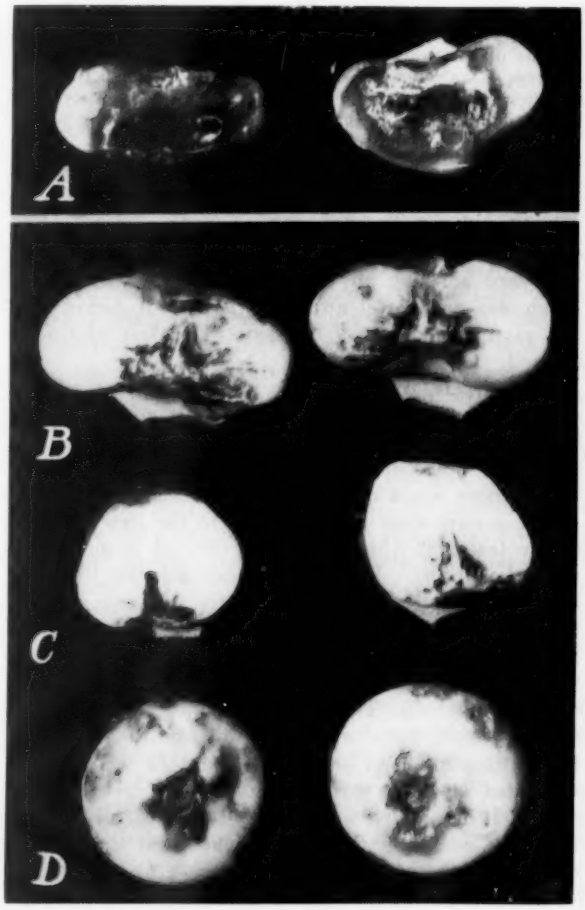


Fig. 1. Gladiolus corms infected with *Urocystis gladiolicola*. A) corms in section showing decay nearly complete. B) and C) corms in earlier stages of the disease. D) bases of 2 corms showing some necrotic tissue at the basal plate, and discoloration apparent through unbroken epidermis. The lesions near the circumference on these corms are due to other causes.

[1] Limber, D. P. 1948. Sori of *Urocystis gladiola* on gladiolus corm scales. Phytopathology 38: 922-925.

[2] MacLean, N. A. 1951. Gladious smut. (Abst.) Phytopathology 41: 941.